Collaborative and Participatory Approaches to Cocoa Variety Improvement

Final Report of the CFC/ICCO/Bioversity International Project on "Cocoa Productivity and Quality Improvement: a Participatory Approach" (2004-2010)

A.B. Eskes, editor

COMMON FUND FOR COMMODITIES

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Bioversity International

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CFC Technical Paper No. 59

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The **International Cocoa Organization (ICCO)** is an inter-governmental Organization composed of 44 exporting and importing Member countries, based in London and established in 1973 under the auspices of the United Nations to supervise and co-ordinate operations in the cocoa and chocolate sector. The mission of the ICCO is to enhance the functioning and sustainability of the world cocoa economy in its social, economic and environmental dimensions through joint co-operation with Member countries and the private sector. Our key objectives are to improve the livelihoods of cocoa farming communities; to increase cocoa productivity and bean quality; to strengthen market transparency; to secure market access for producers; and to address consumer demands related to food quality and food safety.

Bioversity International is part of the Consultative Group on International Agricultural Research, which works to reduce hunger, poverty and environmental degradation in developing countries by generating and sharing relevant agricultural knowledge, technologies and policies. This research, focused on development, is conducted by a Consortium of 15 CGIAR centres working with hundreds of partners worldwide and supported by a multi-donor Fund (http://www.bioversityinternational.org; http://www.cgiar.org; http://cgiarconsortium.cgxchange.org; www.cgiarfund.org). Bioversity seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The organization operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

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Cover illustration
This progressive Venezuelan farmer selected a clone for grafting on his entire farm to improve standing stock. © B. Eskes, Bioversity International/CIRAD.

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PREFACE

Cocoa is produced predominantly by smallholder farmers working on small farms of between 2 and 5 hectares. Productivity on the farms is usually low as a result of production inefficiencies. The main obstacles which prevent farmers from escaping the poverty trap and acquiring the necessary entrepreneurial skills are their lack of formal training in management capability coupled with their limited knowledge of improved production techniques.

World cocoa production increased steadily from ca. 1.5 million tonnes in 1983-84 to a peak of ca. 3.8 million tonnes in 2005-06. This significant increase was almost entirely due to an expansion of production area. However, this approach is unsustainable in the long term as it will lead to deforestation of more conservation areas, thus endangering the environment. A more sustainable way to increase production is to improve farm productivity through the application of best known practices and availability of high-yielding and disease-resistant planting materials.

This Technical Paper is a compilation of studies and experiments conducted in the framework of the CFC/ICCO/26 project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* (2004-2010). The main objective of the project is to improve the welfare of smallholder cocoa farmers through higher and more sustainable productivity levels of good quality cocoa at lower production costs. This is achievable through the selection, distribution and use of new cocoa varieties with improved yield capacity, resistance to pests and pathogens and good quality traits. The project is the logical follow-up to the CFC/ICCO/02 project on *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* (1998-2004), as presented in the CFC Technical Paper No. 50.[1]

Through both projects, a number of cocoa varieties that are high-yielding and pest- and disease-resistant have now been released to cocoa farmers. In addition, a number of new and improved cocoa planting materials are being further tested and validated on research fields for eventual release to farmers.

The Common Fund for Commodities (CFC) and the International Cocoa Organization (ICCO) acknowledge the significant inputs of Bioversity International as Project Executing Agency (PEA) in the successful implementation of the project in 13 countries. In line with the policy to disseminate the information produced by CFC-financed activities, we expect this publication to convey the results and experiences of the project to a wider audience. It is hoped that extension workers, researchers and policy-makers will find the publication to be both useful and relevant for improving the access of cocoa farmers to higher-yielding cocoa varieties with good bean quality and disease resistance.

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Executive Director *a.i.*
International Cocoa Organization

Amb. Ali Mchumo
Managing Director
Common Fund for Commodities

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1 Eskes AB, Efron Y, editors. 2006. Global Approaches to Cocoa Germplasm Utilization and Conservation. Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: a Global Approach" (1998-2004). CFC, Amsterdam, The Netherlands/ICCO, London, UK/IPGRI, Rome, Italy.

FOREWORD

This publication constitutes the proceedings of the Final Workshop of the CFC/ICCO/Bioversity project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* (2004-2010). This workshop was organized by Bioversity and by the Ghana Cocoa Board and held in Accra from 30 May to 4 June 2010. This project was the logical follow-up after the *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* (1998-2004).

The objectives of these projects were:

- Selection of improved varieties with increased yield capacity, resistance to major diseases and pests and with good quality attributes,
- Reinforcement of regional and international collaboration in cocoa breeding,
- Reinforcement of local breeding programmes,
- Direct involvement of farmers in the selection of new varieties through a participatory approach,
- Use of diversity present in international cocoa genebanks to carry out germplasm enhancement for important diseases,
- Distribution of selected germplasm through intermediate quarantine to user countries and through exchange between partners,
- Exchange of information and capacity building.

Key benefits of these projects were:

- Reinforcement of existing cocoa breeding programmes in six countries and re-initiation of breeding programmes in five other countries,
- Selection of new candidate varieties for distribution to farmers,
- Adoption of a farmers' participatory approach through use of farmers' knowledge in selecting promising trees in farmers' fields, and establishment of on-farm trials,
- Establishment of Regional Variety Trials in Africa and in the Americas, aiming at sharing germplasm with disease resistance,
- Evaluation of stability of selection traits through the International Clone Trial established in eight different countries,
- Insights gained in resistance testing methodologies,
- Use of the Trinidad collection to enhance germplasm for black pod and witches' broom resistance,
- Initiation of distribution of selected germplasm through intermediate quarantine (Reading University) to user countries,
- Sensory profiling and independent industry organoleptic evaluations of cocoa liquors made with clones of the International Clone Trial,
- Human capacity building through regular regional and international project workshops and use of project data to obtain university degrees,
- Unprecedented cooperation among research institutes in the cocoa-producing countries, regional and international cocoa research institutes as well as the private sector.

Bioversity International is proud to have been the Project Executing Agency of both projects. It is hoped that the benefits generated by the project will find their way through to the farmers within a few years from now.

Stephan Weise
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Bioversity International
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ACKNOWLEDGEMENTS

The achievements made in the CFC/ICCO/Bioversity project as summarized in this report are to be credited firstly to the scientists and support staff of the 15 collaborating institutions involved (for a full list of institutions see Appendix IV). The technical coordinators of these institutions are to be especially acknowledged: Andrew Daymond and Paul Hadley (University of Reading), Boamah Adomako (CRIG), Bruno Efombagn (IRAD), David Iwaro† and David Butler (CRU), Didier Paulin† and Christian Cilas (CIRAD), François N'Guessan (CNRA), Freddy Amores (INIAP), Jeffrie Marfu and Peter Epaina (CCI), Kelvin Lamin (MCB), Luis Garcia Carrion (UNAS), Patricia Maharaj (MALMR), Peter Aikpokpodion (CRIN), Ventura Gonzalez (INIA), Wilbert Phillips (CATIE) and Wilson Monteiro (CEPLAC).

Special thanks go also to the Directors and Administrative Project Coordinators of all collaborating institutions for their invaluable support to administrative aspects and implementation of the activities.

The CFC/ICCO/Bioversity project was developed under the aegis of the International Cocoa Organization (ICCO) and mainly funded by the Common Fund for Commodities (CFC). However, the implementation of the project would not have been possible without the substantial counterpart and co-financing contributions of all collaborating institutions in the project. The CFC and the co-financing institutions (CRA, CIRAD, Guittard Chocolate Company, Mars Inc., USDA and WCF) are acknowledged for their cash and in-kind contributions, and the national research institutes for their in-kind contributions and dedication to the project activities. ICCO is acknowledged for its continued supervision and political support received during the initiation and implementation to the project.

Special thanks go to:

- Jan Engels, for his positive guidance of the project and for his active participation in project meetings,
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Bertus Eskes
Editor

CONTENTS

INTRODUCTION

This Final Report summarizes the outcomes of the Closing Workshop of the CFC/ICCO/Bioversity project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*, convened at the end of May 2010 in Accra, Ghana. The workshop formally marked the conclusion of the project that was launched in June 2004, but which was building on the achievements of the CFC/ICCO/IPGRI[1] project on *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* implemented between 1998 and 2004. Thus many of the results presented here reflect the work done over a long period of time.

Cocoa breeding during the 1990s had suffered seriously from low cocoa prices. Breeding programmes were underfunded and therefore had reduced their activities or, in some cases, had to cease their activities completely. When the first CFC/ICCO/IPGRI project started, cocoa breeding had come to a virtual stop in five out of the ten countries participating in the project. Meanwhile, destructive diseases and pests had spread, and continued to spread afterwards, to new cocoa-growing areas: witches' broom in Brazil (Bahia), moniliasis in Central and South America, *Phytophthora megakarya* in West Africa (Ghana and Côte d'Ivoire) and pod borer in Indonesia and Papua New Guinea. To find resistant or tolerant planting materials with regard to these pests and diseases was one of the major challenges of both projects.

The second project continued and intensified the on-station selection activities initiated in the first project, and at the same time initiated a farmers' participatory approach. This approach included a farm survey and the direct involvement of farmers in selection of outstanding trees on their farms and in the on-farm trials established between the second and fourth years of the project. The project focused clearly on further capacity building of researchers by linking the research institutes with one another, and through the organization of two project workshops each in the Americas and in Africa in the second and fourth years of the project.

The project's general objectives included:

1. To validate promising cocoa varieties in farmers' fields through participatory approaches, involving farmers directly in the evaluation and selection process;
2. To increase sustainability in cocoa crop improvement programmes through validation and dissemination of selected cocoa varieties between project partners, through enhanced regional and international collaborative research and development activities, and through capacity building;
3. To exchange information and disseminate results among all project partners and also outside the project;
4. To establish and maintain functional linkages between national cocoa breeding programmes, international cocoa genebanks and quarantine centres, and international cocoa research and development efforts.

Promising cocoa varieties have been validated, R&D collaboration enhanced, information generated and exchanged, and networks established. At all project sites, numerous clone and hybrid varieties have been selected for further use in breeding. In five countries new candidate varieties were selected that can be recommended for distribution to farmers. Rapid and/or early resistance screening methods have been validated and successfully adopted for *Phytophthora* pod rot, while screening for resistance to other diseases and pests continues to rely mainly on field evaluations. The International Clone Trial (ICT), planted at eight sites, has yielded important information on stability of agronomic, disease resistance, physiological and

[1] With effect from 1 December 2006, IPGRI and INIBAP operate under the name "Bioversity International", Bioversity for short.

quality traits. The relative stability of these traits over sites suggests that evaluations made at one site will be of value also at other sites. This underpins the value of collaborative approaches in cocoa breeding, such as the germplasm evaluation and enhancement programmes carried out in Trinidad and in Costa Rica.

The work has demonstrated the feasibility of collaborating in cocoa breeding through using similar approaches and exchange of information. The farmers' participatory approach has allowed the farmers to be involved directly in selecting new varieties. In on-farm trials, breeders' selections are being compared to farm selections. This way it is hoped that the farmers adopt materials best suited to their own conditions.

The results of the project were presented in detail during the Project's Closing Workshop (see programme of the workshop, Appendix II) and were described also in the Final Individual Institutes' Reports (see Annexes of the Final Completion Report[2]). The presentations made during the Closing Workshop were included on a CD-ROM and distributed to all participants in July 2010. The detailed presentation of conclusions and recommendations at the end of the workshop are included here as Appendix I.

Several of the activities initiated in the project are still ongoing. Such is the case for the on-farm trials and for the Regional Variety Trials. It is expected that these trials can be continued within the local breeding programmes. However, new collaborative initiatives are required to continue with the pre-breeding programmes and with the distribution of selected materials. The distribution of accessions with resistance to moniliasis and to witches' broom to Africa should continue to be a major objective. Major constraints in cocoa production, such as destructive diseases and pests, vary between the regions (continents). Therefore, continued regional cooperation would be of great value in overcoming these constraints.

Bertus Eskes
Editor

2 Bioversity International. 2010. CFC/ICCO/Bioversity Project: *Cocoa Productivity and Quality Improvement: a Participatory Approach.* Final Completion Report. 1 June 2004 – 30 November 2009. Prepared by Bioversity International, October 2010. (http://www.bioversityinternational.org/fileadmin/bioversityDocs/Announcements/Cocoa_farmers_full_of_beans/CFC-Cocoa_2004-2009_final%20report.pdf).

SUMMARY OF PROJECT RESULTS AND BENEFITS

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Results

Component 1. Participatory approaches to cocoa selection and breeding

Approximately 2000 farms were surveyed in ten different countries. The knowledge of the farmers on their planting materials was documented and results were presented at international cocoa meetings (e.g. INGENIC 2006 Workshop). As planned, approximately 2000 trees were identified as interesting for yield or for low disease or pest incidence. Early screening for *Phytophthora* pod rot (Ppr) resistance carried out in Africa and in Trinidad showed that several of the farmers' selections were highly resistant to the disease. This was in agreement with farmers' knowledge on trees that were identified as less susceptible in farmers' fields.

Approximately 1500 farm selections were established in on-station observation plots or in on-farm trial plots in eight countries. Evaluation of these farm selections has been initiated. Some farm selections appear to be as good as, or better than the local control varieties. Through a co-financing agreement, including with the International Institute of Tropical Agriculture (IITA), the genetic diversity of approximately 2000 farm selections in Africa was analysed using simple sequence repeat (SSR) markers. The results show large genetic variation in the farm population, which is mainly of hybrid origin, with important contributions of the Amelonado, Trinitario and Upper Amazon parental genomes.

Approximately 240 on-farm selection plots were established with support of the project (originally 200 were planned). In most of these plots, varieties selected by breeders are being compared to farm selections (clones or seedling progenies). This activity suffered from drought in Africa and from neglect of some of the farmers. Consequently, the total number of plots that are still actively being observed has been reduced to approximately 120 plots.

Component 2. Collaborative approaches in cocoa breeding

Evaluation and selection of variety trials established in the "Germplasm" project

Approximately 85 ha of variety trials established in the Germplasm project (ICCO/02) have been evaluated throughout the current project. In several countries (e.g. Brazil, Ghana, Nigeria, Papua New Guinea and Trinidad and Tobago) several new varieties were selected or confirmed for commercial distribution to farmers. Numerous promising individual trees or hybrid varieties were selected to be used in further confirmation trials in several other places (e.g. Brazil, Ecuador, Côte d'Ivoire and Venezuela). New variety trials with promising selections were established in Brazil, Côte d'Ivoire, Ecuador, Malaysia and Papua New Guinea.

The International Clone Trial, established in eight countries, has also been evaluated throughout the second project. Although some clones yielded quite well, in general the average of the local control clones evaluated in the Local Clone Trial yielded substantially more than the average of the International Clones. This shows the low level of adaptation of many of the International Clones, which were not previously selected for yield potential.

However, some of the International Clones out-yielded the local control clones at a few sites. Evaluation of sensory quality was carried out for the International Clones during 2007-09. Cocoa liquors of approximately 200 cocoa bean samples were prepared and distributed by Guittard Chocolate Co. to three panels (Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France; Cocoa Research Unit (CRU), Trinidad and Tobago; and Guittard/Mars Inc., USA). Data analyses showed significant environmental effects for cocoa flavour, acidity and astringency and clone effects for floral flavour. Interactions between environment and clones estimated over 2 years of evaluations were not significant, whereas significant effects were obtained for individual years. Significant differences between clones were also observed for agronomic traits at all sites. Yield data appeared to be significantly correlated over a large number of sites, while vigour was less well correlated between sites. Physiological traits varied over sites and clones reacted variably, suggesting that different genotypes require different management practices (such as pruning) to optimize yield performance.

Regional Variety Trials (RVTs)

The project supported the establishment, between 2005 and 2006, of an RVT in the Americas (six countries) and of another RVT in Africa (four countries). The objective was to exchange hybrid varieties with good yield potential and with resistance to diseases (Ppr, moniliasis and witches' broom). The first results in Costa Rica show significant variation in resistance to moniliasis (10-50% infection), with two hybrids having very low infection levels.

Germplasm enhancement for disease resistance

A large Germplasm Enhancement programme for resistance to Ppr has been implemented at CRU already from 1998 onwards. Interesting results were observed for the Ppr resistance enhancement, obtaining approximately 70% of resistant (R) or moderately resistant (MR) trees after one cycle of selection of crosses among selected parental accessions of the International Cocoa Genebank, Trinidad and Tobago (ICG,T). The original population only contained 30% of MR or R trees. CRU already initiated the second cycle, i.e. selection of seedlings from crosses between first-cycle selections. A similar enhancement programme for resistance to witches' broom was initiated in 2004. Approximately 5000 seedlings were evaluated and 200 selected seedlings (also tested for resistance to Ppr) were planted in the field for further observations.

Quarantine and distribution of selected accessions

The so-called "CFC/ICCO/Bioversity Collection" was selected as part of the first project activities. This collection contains 112 accessions mainly selected from the ICG,T for resistance to Ppr, but it also contains selections with resistance to witches' broom and to moniliasis. During the current project, this collection was sent to the Reading University intermediate quarantine facility, where it underwent virus indexing for a 2-year period. At the end of the project, 80 clones had completed quarantine. Distribution of this collection has been initiated, especially to African countries.

Resistance testing methods

The use of the leaf disc and pod tests for Ppr resistance screening was validated during the second project in Côte d'Ivoire and in Cameroon.

Screening for resistance to witches' broom with the traditional spray method had shown low consistency at individual plant level. In Trinidad, good results were reported by using the agar-droplet method, aiming at high infection level and evaluation of the broom base diameter as the main selection trait. This method was since adopted for individual seedling selection in the germplasm enhancement programme. Pod inoculations were tried but infection levels were low or inconsistent. More work will be required to improve this method.

Early screening for resistance to moniliasis by inoculation of seeds or young seedlings failed to produce symptoms. The pod inoculation method in the field proved to be reliable, and this was used successfully in Costa Rica as well as in Ecuador.

Testing of antixenosis (choice of insect to feed on twigs from different cocoa genotypes), tolerance and antibiosis (capacity to survive on different host tissues) to cocoa mirids showed often inconsistent results and the implementation of these methods is laborious. However, antixenosis appeared to be related to long-term damage of mirids observed in Côte d'Ivoire. *Lasiodiplodia* was easily isolated from young and old mirid wounds, showing a possible association with mirid damage. Screening methods for *Lasiodiplodia* resistance were tested using *Lasiodiplodia* inoculations of wounded seedlings and detached twigs. Results were inconsistent, including when artificial wounding was used as a method to facilitate natural infection with *Lasiodiplodia.*

Main benefits

- Reinforcement of existing cocoa breeding programmes in 11 countries.
- Selection of 55 new candidate varieties for distribution to farmers in Brazil, Ecuador, Nigeria, Papua New Guinea and Trinidad and Tobago.
- Selection of numerous varieties to be used in further breeding (all cocoa-producing countries).
- Adoption of a farmers' participatory approach in cocoa breeding through capturing farmers' knowledge on their planting materials, selection of interesting trees and establishment of on-farm trial plots.
- Establishment of two Regional Variety Trials in six countries in the Americas and in four countries in Africa, aiming at sharing of varieties with disease resistance.
- Evaluation of stability of cocoa traits through the International Clone Trial, using similar evaluation methods.
- Insight has been gained into resistance testing methods. Positive results were obtained with testing for Ppr resistance methods, whereas less consistent results were obtained with methods used for other diseases and pests.
- Use of the Trinidad germplasm collection to carry out pre-breeding for resistance to *Phytophthora* pod rot and to witches' broom disease.
- Initiation of distribution of germplasm selected in the project through quarantine at the Reading University to user countries, especially African countries.
- Unprecedented cooperation was achieved among research institutions in the cocoa-producing countries, regional and international institutions, and the private sector.
- The project has positively impacted on cocoa breeding programmes, through reinforcement of ongoing breeding programmes.
- A total of 1500 promising trees were identified by using a farmers' participatory approach.
- More than 100 selected genotypes were quarantined and distribution to user countries was initiated.
- Human capacity building was achieved through the organization of four regional workshops and exchange of results (publications, project reports).
- The data generated in the project were used to obtain three PhD degrees in Africa, and several MSc and undergraduate degrees elsewhere.
- As a spin-off of the project, institutes have managed to obtain new projects in cocoa breeding and also in other cocoa research areas.

COUNTRY REPORTS

On-farm and on-station selection of new cocoa varieties in the State of Bahia, Brazil

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Introduction

Cocoa production constraints

The selection of more productive and resistant varieties and their availability to the cocoa producers have been among the bottlenecks for this culture in Brazil, especially in the State of Bahia. Without these planting materials, the local farms cannot develop economically in a sustainable manner. Witches' broom (WB) disease was introduced in Bahia in 1989, causing enormous losses to the local economy due to the difficulty of its control. The use of resistant varieties has been chosen as one of the solutions for the local cocoa culture. The cocoa populations in the farms represented a potential source for identification and selection of productive and resistant clones. Therefore producers, cocoa breeders and extension agents of the Centro de Pesquisas do Cacau (CEPLAC) have been working in a participatory way during the last 15 years, approximately, on the identification and selection of planting materials for the substitution of decadent cocoa plantations and establishment of new ones. CEPLAC has also been developing a variety improvement programme focusing on clone selection, and populations are being genetically improved looking for the pyramiding of genes associated with resistance to WB, yield, quality and self-compatibility. The lack of information on the correct cocoa husbandry practices has also prevented the producers to obtain sufficiently high levels of productivity, even when using good varieties. Consequently, most cocoa plantations became low productive areas due to the malformation of the plants and excessive shade.

Cocoa breeding objectives

The main objective is the development of clones and/or progenies with good yield and quality characteristics, self-compatibility and broad resistance base to WB and *Phytophthora* pod rot (Ppr).

Current status of cocoa varieties

CEPLAC has recommended for planting about 38 cocoa clones, but some of them have become susceptible to WB. Besides, some clones are self-incompatible and the producers have shown their preference for self-compatible clones. The cocoa breeders and producers are always searching for new superior genotypes, and a great number of clones has been selected locally. The most promising selections are being tested in several farms, in a participatory approach. Many of these materials were also transferred to other States of the Union, such as Espírito Santo and Pará.

Farmer participatory selection activities

Farm surveys

- **Simplified model for clone evaluation**

In Bahia, cocoa farmers have been selecting plant materials in their populations for a long time. CEPLAC's cocoa breeders had taken the initiative of selecting plant materials resistant to WB in the farm populations. Selected plants were cloned and established in the field as observation plots, in a farm called "Rainha do Sul". This farm became an important visiting place for the cocoa producers, extension agents, governmental authorities and financing institutions. It also became a platform for the diffusion of technologies related to clone selection, selection criteria, propagation and plant management, encouraging the cocoa producers to renew their plantations and to participate in the selection programme (Lopes et al. 2005). Large quantities of planting materials were afterwards selected by the cocoa producers under the guidance of CEPLAC. Many of these selections are also being tested in multilocation trials, which are usually very expensive and demanding in time. For these reasons, a simplified model for evaluating a large number of clones is being implemented. About 50 farms distributed in the cocoa region are participating in an evaluation network that began with clones already in production chosen by producers, farm managers and extension agents, according to their criteria, among the varieties indicated by Centro de Pesquisas do Cacau (CEPEC)/CEPLAC and farm selections. The participating farms are also receiving clones selected in the breeding populations of CEPEC and the producers feed the evaluation network with data taken from the plants. The data from 45 clones that are being evaluated in six distinct farms of the group M. Libânio Agricola SA were analysed (Table 1) and the results showed that the productivity mean of the clones recommended by CEPLAC was 64.9±6.4 @/ha (1@=15 kg) in the main harvest season and 27.4±4.6 @/ha in the secondary harvest season, during the period from 2004 to 2007 (plants aged 2 to 9 years). For the group of remaining clones, the mean yield was 74.8±14.0 @/ha in the main harvest and 46.0±14.6 @/ha in the secondary harvest. The methodology used allows the isolation of the effects due to age of plants and year of evaluation, enabling a more adequate interpretation of data with respect to the age of stabilizing yield. The average yield of the incompatible clones was 38% lower than that of the compatible clones.

Farm selections

- **Amazon collecting expedition**

Cocoa breeders at CEPLAC have been constantly searching for new sources of resistance and productivity. During the period 6-12 May 2007 a cocoa collecting expedition was carried out in the Middle Amazon, in an area comprised between the municipal districts of Urucurituba (2°47'S, 57°20'W) and Itacoatiara (3°8'S, 58°25'W) on the margins of the Amazon River. The area contains very old traditional cocoa cultivated in the high flood lands (*varzeas*). The cocoa is semi-domesticated as it has been improved by the local communities for more than three centuries, by employing solely the process of phenotypic selection. Those populations have been very little explored and represent a great potential for breeding, possibly because of the high level on endogamy. These varieties show many important well-fixed traits and are well adapted to the *varzea* conditions. They are quite productive and they also seem to be resistant to local diseases. Prevailing variations in pod size and shape are shown in Figure 1.

During the expedition, seed and pod measurements were taken from all selected mother trees. Seeds or budsticks from 87 mother trees were collected, resulting in 17 clones and 86 open-pollinated progenies.

Table 1. Corrected productivity means of different clones per season and per year (main harvest, secondary harvest and annual) and the annual real mean, expressed in @/1000 plants (1@=15 kg)

Clone	Corrected mean				Real mean (annual)
	Main harvest	Secondary harvest	Annual		
CEPEC 42	61.96	12.58	74.54		81.00
EET 397	45.81	12.27	58.07		67.00
TSH 1188	37.31	8.48	45.79		51.15
TSH 516	54.84	5.82	60.67		69.64
TSH 565	39.71	5.95	45.66		56.01
Mean			**56.95**		**64.96**
TSA 654	36.94	9.08	46.01		52.77
TSA 656	26.30	3.64	29.94	**	45.96
TSAN 792	67.49	9.66	77.15	**	73.28
TSH 774	27.64	4.30	31.94	**	40.72
VB 1151	36.82	74.59	111.41	**	103.70
VB 514	42.62	10.93	53.55		60.17
VB 547	45.04	8.70	53.74		68.12
VB 681	20.98	41.95	62.92		68.50
VB 900	43.09	2.00	45.09		64.71
CCN 10	71.03	37.23	108.26	**	106.62
CCN 51	47.14	60.90	108.04	**	112.84
PH 16	67.06	41.91	108.97	**	106.16
PS 1319	38.25	96.41	134.66	**	125.89
RVID 08	26.31	16.00	42.32		50.26
SJ 02	18.87	52.27	71.15		66.14
VB 276	72.20	-1.35	70.85		98.92
VB 515	84.39	9.93	94.32	**	96.79
BJ 11	76.66	51.88	128.53	**	117.05
CAMACA 1	53.85	7.66	61.52		61.29
CCN 16	72.53	18.55	91.09	*	88.34
CEPEC 515	50.82	23.36	74.17		66.02
IPIRANGA	50.57	9.26	59.83		67.78
LP 06	73.61	70.61	144.23	**	144.84
MAT 16	54.15	27.93	82.08		76.08
PAIN 9316	40.27	70.41	110.68	**	107.55
PH 09	37.12	71.39	108.51	**	115.17
PH 114	59.45	42.46	101.91	**	119.15
PH 129	107.59	33.82	141.41	**	151.92
PH 15	38.87	82.38	121.24	**	120.06
PH 92	17.56	55.74	73.29		94.67
PS 1030	89.70	36.00	125.71	**	140.81
PV 7006	49.98	8.52	58.50		79.18
SAL 03	38.10	51.86	89.96	**	83.77
VB 1152	44.31	16.51	60.82		57.69
VB 1156	17.89	60.93	78.82		69.19
VB 566	56.95	6.06	63.01		65.48
VB 663	41.08	12.00	53.09		57.71
VB 902	37.82	13.82	51.64		54.11
VB 979	75.43	4.37	79.80	*	107.87
VB 990	90.72	-2.23	88.48	**	116.55

** and * = significance for P=0.01 and P=0.05, respectively, for the contrast between means of each clone and the mean of the first five clones recommended by CEPLAC.

Figure 1. Example of the variability in the cocoa populations of the Middle Amazon, Amazon, Brazil.

The seedling progenies were established in the field according to a completely randomized block design, with three replications and eight plants per plot. Plants are still very young, but some disease evaluations have been made, especially related to WB. Table 2 and Figure 2 show results of the incidence of WB on the seedling accessions in the collection established in Ilhéus, Bahia. Most progenies presented a high percentage of plants with symptoms, but there is also a group of accessions with a low percentage of infected plants, which may allow for the selection of new sources of resistance.

Table 2. Number of plants (#P), number of vegetative brooms (VB) and percentage of dead plants (%DP) for each accession of the Amazon collection, CEPEC, Ilhéus, Bahia

Accession code	#P	VB	%DP
AM 1064	24	17	20.8
AM-1065	16	15	6.3
AM 1066	48	28	29.2
AM 1067	24	8	20.8
AM 1068	40	22	15.0
AM 1069	24	2	58.3
AM 1070	24	4	33.3
AM 1071	40	18	22.5
AM 1072	48	16	27.1
AM 1073	24	8	8.3
AM 1075	24	6	37.5
AM 1076	24	17	45.8
AM 1077	16	6	0.0
AM 1079	40	36	25.0
AM 1081	48	37	18.8
AM 1083	16	13	18.8
AM 1085	24	15	4.2
AM 1086	40	27	27.5
AM 1088	40	27	12.5
AM 1091	24	2	33.3
AM 1092	40	12	17.5
AM 1093	48	55	14.6
AM 1095	32	20	31.3
AM 1097	40	26	12.5
AM 1099	24	12	4.2
AM 1100	24	12	25.0
AM 1101	40	24	12.5
AM 1103	24	22	29.2
Control	16	0	0.0
AM 1105	40	30	7.5
AM 1107	40	27	20.0
AM 1109	24	16	16.7
AM 1111	16	6	18.8
AM 1112	32	35	31.3
AM 1114	24	10	20.8
AM 1115	48	33	20.8
AM 1116	24	10	20.8
AM 1117	32	16	18.8
AM 1118	24	8	33.3
AM 1119	24	18	12.5
AM 1120	40	22	30.0
AM 1121	24	4	25.0
AM 1122	24	16	37.5
AM 1123	40	11	40.0
AM 1124	24	4	33.3
AM 1125	32	8	31.3
AM 1126	24	18	8.3
AM 1127	40	40	17.5
AM 1128	8	9	12.5
AM 1129	16	4	6.3
AM 1130	24	14	29.2
AM 1131	24	4	37.5
AM 1132	32	16	34.4
AM 1133	40	41	7.5
AM 1134	32	25	25.0
AM 1135	40	52	12.5
AM 1136	24	26	20.8
AM 1137	40	30	15.0
AM 1138	24	19	4.2
AM 1139	24	7	16.7
AM 1140	40	16	40.0
AM 1141	40	37	15.0
AM 1142	24	10	29.2
AM 1143	40	34	32.5
AM 1144	24	10	25.0
AM 1145	16	6	25.0
AM 1146	24	9	12.5
AM 1147	24	8	12.5
AM 1148	24	12	20.8
AM 1150	40	13	25.0
AM 1151	24	16	8.3
AM 1152	24	7	45.8
AM 1153	24	24	12.5
AM 1154	24	10	16.7
AM 1155	24	6	54.2
AM 1157	24	4	8.3
AM 1158	24	1	41.7
AM 1159	24	10	16.7
AM 1160	24	7	54.2
AM 1161	24	2	25.0
AM 1162	24	18	16.7
AM 1163	24	23	25.0
AM 1164	24	14	20.8
AM 1165	24	16	12.5
AM 1166	24	9	25.0

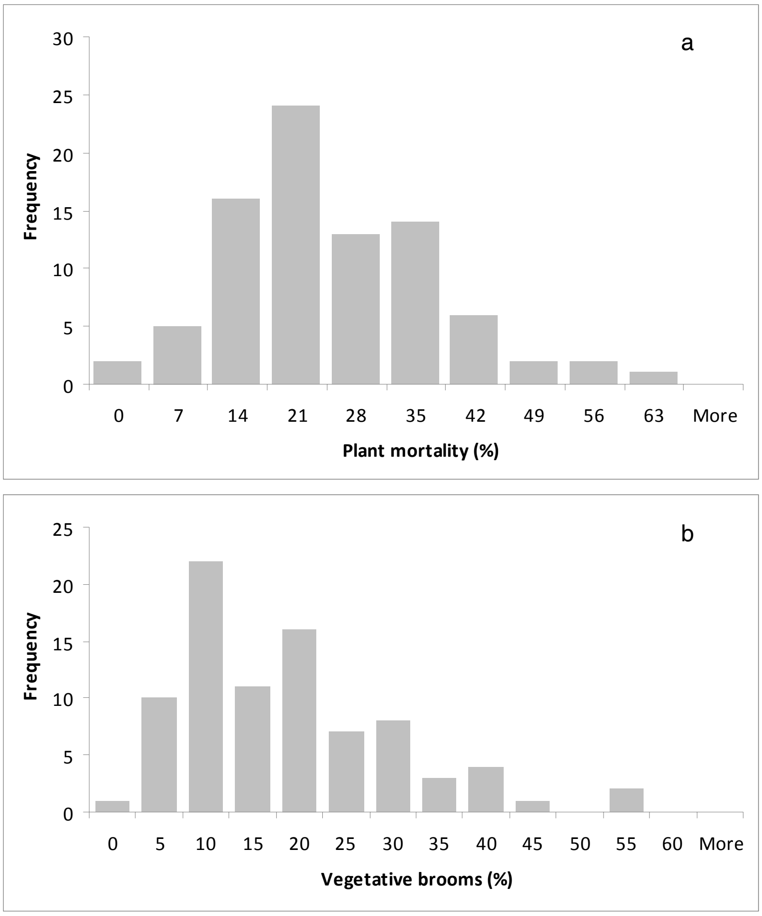

Figure 2. Amazon collection distribution with respect to (a) percentage of dead plants and (b) percentage of vegetative brooms at CEPEC, Ilhéus, Bahia.

On-farm trials

- **Multilocation on-farm variety trials**

Clones that were selected in the breeding populations and also those selected in the farm populations by the farmers are being tested in different locations (farms), in the cocoa region of Bahia. These multilocation clone trials, all following a completely randomized design, were grouped as follows:

- Group 1: 25 clones, with 20 plants per clone in 3 localities
- Group 2: 30 clones, with 20 plants per clone in 4 localities
- Group 3: 30 clones, with 20 plants per clone in 4 localities
- Group 4: 100 clones, with 20 plants per clone in 5 localities
- Group 5: 45 clones, with 20 plants per clone in 3 localities.

Clones TSH 1188 and SCA 6 were included as resistance controls, and SIC 23 and SIAL 169 as susceptible controls.

In all trials the clones were grafted on basal chupons of the old cocoa trees, preferentially. However, many cocoa plants did not present such basal chupons in condition to be grafted. Besides, many plants only produced lateral chupons. Thus these differences related to chupons such as age, development and site of insertion, not only concurred to increase the clonal variation, but also the competition between plants, resulting in a heavy mortality of plants and a drop in yield.

The variables scored in these clone trials were: number of vegetative brooms (VB), number of cushion brooms (CB), number of pods attacked by *Phytophthora* (Ppr), number of pods attacked by WB (WBAP), number of pod and cherelles (#P&C) and the dry seed production per plant.

Group 1: farms of Santa Ursula, Corcovado and Conceição
The clone trials were concluded in 2007. The data analyses showed significant differences for the effects of year, clone and the interaction clone x year for all traits, at the probability level of 0.05. The variation within clone is rather high, as indicated in Figure 3. The complexity of the interaction between clones and rootstocks (chupons) is notable, hindering, thus, the true potential of the clones. For this reason it was decided to exclude from the analyses all plants with very poor field performance due to more serious malformation problems, e.g. those related to rooting. In this group, the clones CCN 51 (CP 01), CP 02 (CCN 10), CCN 16 (CP 04), CP 06 (EET 392), CP 14 (VB 514), CP 16 (VB 681), CP 15 (VB 663), CP 19 (VB 557), CP 20 (VB 892) and CP 23 (VB 900) outperformed the control varieties in terms of resistance to WB and seed production and all are being recommended by CEPLAC for planting. The VB selections represent farm selections.

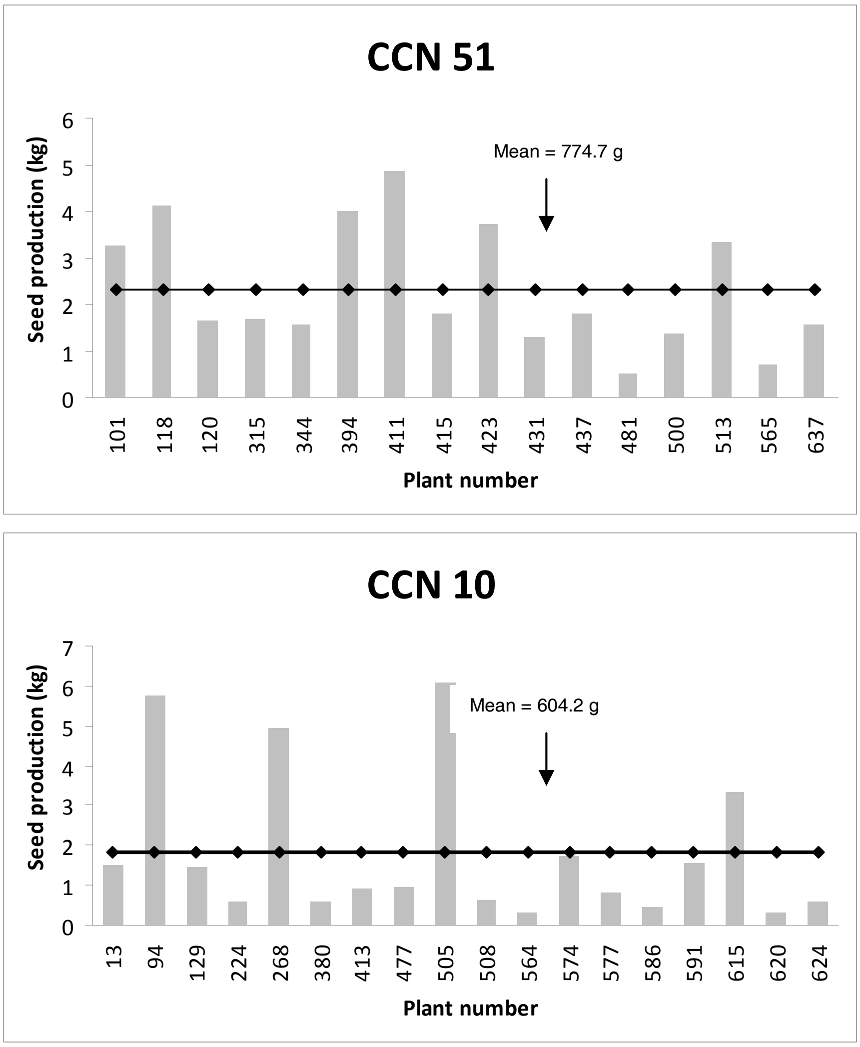

Figure 3. Observed variation within clone for seed production.

Group 2 (MLCT-02): farms of Joventina, Nova Tranquilidade, Santa Helena and Monte Alegre

Most clones derived from the new breeding populations produced in the framework of the CFC/ICCO/IPGRI project. The Nova Tranquilidade trial had to be cancelled because the producer was not dispensing adequate cultural practices, the soil conditions were not good at all and the mortality of plants was excessive. The data analyses by location showed significant differences for the effects of year, clone and the interaction clone x year, for all traits, at the probability level of 0.05. The variation within clones was again quite large and may be attributed to the complexity of the interaction between clone and rootstocks. It was observed that for the traits related to disease resistance, the CP clones either outperformed the resistant control varieties or remained at the same level. In the Santa Helena farm, the means for dry seed weight per plant and per clone ranged from 173.7 to 975.0 g and the coefficient of variation ranged from 75.7 to 159.9%. In the Joventina farm, the ranges observed were 85.0 to 1662.3 g for seed production and 48 to 192% for the coefficient of variation; for the Monte Alegre farm the values were 50.2 to 1120.7 g for the seed production and 45 to 156% for the coefficient of variation. Also, in terms of yield, many clones outperformed the control clones: among them are CP 25, CP 45, CP 46, CP 47, CP 49, CP 50, CP 53, CP 55 and CP 56. The clones CP 25, CP 46, CP 49, CP 50 and CP 55 are being recommended by CEPLAC for commercial planting.

Group 3: farms of Belo Horizonte, Nova Vitoria, Lagoa Grande and Porto Seguro

This group included mainly clones selected in the hybrid trials that were established a long time ago in the Experimental Station of Filogônio Peixoto (ESFIP), Linhares, ES, coded as PR selections. Most of these clones are self-incompatible. In general, the field performance of the clones was very poor and the analyses of variance showed that the effects of year, clone and the interaction clone x year were significant in most of occasions, except for dry seed yield in the farms Belo Horizonte, Lagoa Grande and Nova Vitoria. Also, a large variation was observed within clones as a consequence of the numerous factors related to the development of grafted chupons.

In Belo Horizonte, the clone means for dry cocoa yield ranged from 47.3 to 241.0 g/plant and the coefficient of variation ranged from 79.6 to 181.2%; the means for the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 97.8, 74.2, 41.9 and 117.6 g/plant, respectively.

In Lagoa Grande, the clone means ranged from 179.0 to 920.7 g/plant and the coefficient of variation ranged from 48.0 to 120.4%; the means for the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 298.0, 179.0, 200.9 and 747.8 g/plant, respectively.

In Nova Vitória, the clone means ranged from 128.1 to 750.2 g/plant and the coefficient of variation ranged from 54.1 to 120.4%; the means for the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 411.0, 128.0, 137.0 and 375.0 g/plant, respectively.

In Porto Seguro, the mean values ranged from 105.0 to 289.0 g/plant, with coefficients of variation ranging from 92.3 to 140.1% and those of the control varieties SCA 6, SIAL 169, SIC 23 and TSH 1188 were 286.0, 148.0, 105.9 and 283.5 g/plant, respectively. However clones such as PR 16, PR 23, PR 26 and PR 55 were able to produce more than 1200 g of dry seeds per plant with low disease incidence.

Group 4: farms of Barra do Rocha, Calumbi, CEPLAC (Estação Experimental Arnaldo Medeiros, ESARM), Corcovado, Maia and Venturosa

The data collection was done monthly from 2002 to 2008. It was observed that most of the clones presented levels of resistance similar or superior to TSH 1188 (resistant control), which presented average numbers of 1.8 vegetative brooms (VB) and 4.6 cushion brooms

(CB), while for SIAL 169 and SIC 23 (susceptible controls) these numbers were 38.4 and 35.7 for VB and 8.2 and 4.2 for CB.

Some of the clones of this trial were also tested for resistance to *Ceratocystis* wilt. Sixty-four clones were inoculated with a spore suspension of *C. cacaofunestra*, directly in the field. The area of lesions was measured and the results are presented in Table 3. A wide variation in the size of lesions was observed. The clone TSH 1188 presented the smallest lesion area (2.19 mm^2), but other clones also presented small lesions, indicating a good level of resistance and potential to be used as a source of resistance in the breeding programme.

The accumulated yield was estimated in terms of the dry seed weight per clone per hectare over the period of 2003 to 2008. The results presented in Table 4 showed that, in general, the clones performed better in the Corcovado and Maia farms. It was observed that more than 47 clones outperformed the TSH 1188 (mean = 1249.5 g) and that five clones had means superior to 2500 g (F1 04, BB 6018, PS 13.19, VB 311 and SP 50). Among these 47 clones are included also some farm selections that are recommended by CEPLAC, such as SJ 02, VB 515, VB 547, VB 679, RVID 08, PH 16 and PS 13.19. The fat content was also determined for 66 clones (Table 5) and ranged from 49.8% to 56.8%. Nine clones presented percentages equal or superior to TSH 1188 (55.8%) and eight other clones recommended by CEPLAC and also tested in these trials presented fat contents ranging from 50.9% to 53.8%, which are superior to the local clones SIC 23 and SIAL 169.

Table 3. Area of lesion associated with the *Ceratocystis* infection in 64 cocoa clones inoculated in the field (CEPEC trial)

Clone	Area of lesion (mm^2)
AM 01	47.40
AM 02	36.14
BB 1.33	2.60
BB 6020	22.76
BB 6021	14.51
DF 01	26.34
FADA 100	35.36
FADA 18	33.05
FF 38	17.57
FL 16	29.23
FL 29	29.17
FL 60	5.08
FL 77	14.15
FLN 30	14.10
FLN 46	8.09
FSR 01	7.56
FSU 01	23.51
FSU 07	27.09
FSU 127	43.28
FSU 13	7.92
FSU 151	14.25
FSU 77	14.48
GM 33	8.07
HR 20	5.11
HR 29	19.47
M 05	16.33
NV 02	27.56
NV 22	10.68
NV 77	12.60
PAT 118	4.45
PAT 84	36.65
PB 617	8.46

Clone	Area of lesion (mm^2)
PH 16	41.19
PS57 1	20.11
PS57 111	22.58
RT 09	18.46
RT 106	24.76
RV-ID 12	16.68
SCS 18	5.46
SCS 20	3.49
SIAL 169	19.31
SIC 23	6.56
SJ 02	27.85
SM 02	26.10
SM 04	27.49
SM 06	32.19
T 10	22.60
T 11	13.89
TR 12	16.53
TR 15	20.27
TR 22	8.42
TR 35	28.58
TR 36	14.15
TSH 1188	2.19
VB 309	12.90
VB 311	26.36
VB 316	55.98
VB 515	17.68
VB 547	23.17
VB 679	30.31
VB 892	42.84
VB 900	25.33
VB 902	6.87
VB 903	9.36

Table 4. Accumulated yield of dry beans (kg/ha) for the period from November 2003 to February 2008 in the farms of Barra do Cedro, Calumbi, CEPEC, Corcovado, Maia and Venturosa, and mean yield for each clone over the farms where it was tested

Clone	Dry bean yield (kg/ha)						Mean yield (kg/ha)
	Barra do Cedro	Calumbi	CEPEC	Corcovado	Maia	Venturosa	
FL 77	-	27.0	499.5	529.5	-	-	352.5
T 11	456.0	544.5	1420.5	-	883.5	282.0	717.0
FADA 18	-	-	795.0	-	-	-	795.0
BB 6021	-	-	889.5	-	-	-	889.5
BB 6020	156.0	372.0	1545.0	1869.0	1093.5	360.0	900.0
T 10	-	123.0	1609.5	-	1357.5	513.0	901.5
FADA 100	289.5	-	1285.5	-	1704.0	357.0	909.0
SM 06	342.0	1012.5	1122.0	1843.5	-	273.0	919.5
TR 15	129.0	252.0	1056.0	2550.0	1284.0	291.0	927.0
PB 617	303.0	1117.5	679.5	1548.0	1300.5	651.0	933.0
PS57 1	-	-	1446.0	-	-	448.5	946.5
SM 02	421.5	958.5	816.0	1582.5	1353.0	598.5	955.5
M 04	190.5	651.0	-	2452.5	1218.0	321.0	966.0
M 05	139.5	241.5	979.5	2485.5	1165.5	783.0	966.0
VB 900	372.0	483.0	736.5	1756.5	1926.0	534.0	967.5
SM 12	457.5	-	-	-	1806.0	643.5	969.0
FSU 01	375.0	262.5	1110.0	3183.0	1021.5	318.0	1045.5
VB 903	-	-	1068.0	-	-	-	1068.0
SCS 18	-	-	1051.5	-	1092.0	-	1071.0
GM 33	-	763.5	1359.0	-	1711.5	511.5	1086.0
CSF 22	909.0	-	583.5	-	2229.0	651.0	1093.5
VB 892	309.0	792.0	1806.0	1894.5	1515.0	444.0	1126.5
NV 77	-	-	2157.0	-	-	100.5	1128.0
VB 902	237.0	537.0	1968.0	1870.5	1833.0	375.0	1137.0
PAT 118	706.5	-	1446.0	1887.0	1369.5	411.0	1164.0
PH 109	-	765.0	-	1641.0	-	-	1203.0
LP 24	-	652.5	-	2790.0	-	187.5	1210.5
PAT 84	552.0	-	1563.0	2161.5	1579.5	306.0	1231.5
VB 679	448.5	1191.0	1224.0	2242.5	1725.0	799.5	1272.0
AM 02	1117.5	798.0	-	2961.0	-	243.0	1279.5
FLN 46	-	-	1285.5	-	-	-	1285.5
SM 04	199.5	456.0	1884.0	2128.5	2479.5	562.5	1285.5
RVID 12	495.0	582.0	1366.5	2719.5	2020.5	588.0	1294.5
SCS 20	-	-	1108.5	-	1492.5	-	1300.5
RT 106	624.0	-	1725.0	-	2496.0	495.0	1335.0
DF 01	-	-	1365.0	-	-	-	1365.0
FF 38	412.5	-	1365.0	3205.5	1464.0	490.5	1387.5
SIAL 169	**36.0**	**457.5**	**1189.5**	**1473.0**	**384.0**	**190.5**	**622.5**
SIC 23	**25.5**	**240.0**	**958.5**	**2713.5**	**304.5**	**129.0**	**729.0**
TSH 1188	**606.0**	**1050.0**	**1306.5**	**2344.5**	**1765.5**	**429.0**	**1249.5**

Table 4 (continued). Accumulated yield of dry beans (kg/ha) for the period from November 2003 to February 2008 in the farms of Barra do Cedro, Calumbi, CEPEC, Corcovado, Maia and Venturosa, and mean yield for each clone over the farms where it was tested

Clone	Dry bean yield (kg/ha)						Mean yield (kg/ha)
	Barra do Cedro	Calumbi	CEPEC	Corcovado	Maia	Venturosa	
PS57 111	-	-	1207.5	2181.0	1668.0	514.5	1392.0
NV 22	937.5	747.0	1714.5	-	3301.5	276.0	1395.0
NV 00	558.0	-	-	-	3381.0	336.0	1425.0
FL 16	595.5	-	1572.0	2433.0	2127.0	408.0	1426.5
AM 01	-	-	1173.0	2193.0	1440.0	987.0	1449.0
BB 1.33	390.0	777.0	1734.0	-	3478.5	1074.0	1491.0
FSU 77	691.5	1095.0	1011.0	3877.5	1668.0	612.0	1492.5
RT 15	-	1110.0	-	2001.0	-	-	1555.5
BB 6030	561.0	1390.5	-	2947.5	2520.0	363.0	1557.0
FC 328	219.0	-	-	3312.0	1153.5	-	1561.5
LP 30	-	1000.5	-	3373.5	1453.5	469.5	1573.5
FL 44	949.5	-	-	2272.5	-	-	1611.0
VB 309	-	-	1678.5	1554.0	-	-	1615.5
RVID 08	972.0	2694.0	-	-	-	1251.0	1638.0
HR 29	-	-	1660.5	-	-	-	1660.5
VB 515	978.0	1330.5	1087.5	3132.0	3022.5	592.5	1690.5
FC 16	655.5	-	-	3249.0	1200.0	-	1702.5
TR 12	364.5	-	2020.5	3436.5	2142.0	732.0	1740.0
FSU 13	61.5	1597.5	1434.0	4287.0	2319.0	982.5	1780.5
FSU 151	471.0	1747.5	1911.0	2910.0	3436.5	709.5	1864.5
PH 16	51.0	-	1623.0	4785.0	-	1072.5	1882.5
TR 35	114.0	399.0	2458.5	3024.0	3427.5	-	1884.0
HR 20	-	-	1897.5	-	-	-	1897.5
SJ 02	786.0	-	844.5	5776.5	1587.0	537.0	1906.5
VB 547	775.5	1239.0	1486.5	3744.0	4120.5	336.0	1950.0
RT 09	748.5	1578.0	1507.5	3795.0	3055.5	1147.5	1972.5
FLN 30	1218.0	-	2785.5	3216.0	2395.5	724.5	2067.0
F1 17	-	669.0	-	3418.5	2331.0	-	2139.0
NV 21	-	1291.5	-	3075.0	-	-	2184.0
FO-30 9	-	1123.5	-	3391.5	-	-	2257.5
FL 60	985.5	-	2463.0	4746.0	2265.0	1020.0	2295.0
RT 06	735.0	2056.5	1986.0	5797.5	2668.5	822.0	2344.5
FSR 01	-	-	2473.5	-	-	-	2473.5
F1 04	-	-	-	2641.5	-	-	2641.5
BB 6018	-	1300.5	-	6136.5	2863.5	334.5	2659.5
PS 13.19	-	589.5	-	4761.0	-	-	2674.5
VB 311	-	-	2400.0	3391.5	-	-	2896.5
SP 50	-	-	-	4780.5	1219.5	-	3000.0
SIAL 169	**36.0**	**457.5**	**1189.5**	**1473.0**	**384.0**	**190.5**	**622.5**
SIC 23	**25.5**	**240.0**	**958.5**	**2713.5**	**304.5**	**129.0**	**729.0**
TSH 1188	**606.0**	**1050.0**	**1306.5**	**2344.5**	**1765.5**	**429.0**	**1249.5**

Table 5. Butter content for the clones of the Group 4 trials

Clone	Butter content (%)	Clone	Butter content (%)	Clone	Butter content (%)
AM 02	51.5	HR 20	56.6	T 10	56.2
BB 1.33	56.8	HR 29	50.9	T 11	52.2
BB 6020	56.4	M 05	54.5	TR 12	54.7
BB 6021	53.1	NV 22	55.0	TR 15	49.8
DF 01	54.6	NV 77	52.6	TR 22	54.6
FADA 100	53.3	NVSV 1	52.2	TR 35	51.2
FADA 18	55.6	PAT 84	55.8	TR 36	53.9
FF 38	55.6	PB 617	54.9	VB 276	52.6
FL 16	52.6	PH 16	50.9	VB 309	56.2
FL 29	56.2	PS 1319	52.6	VB 311	53.7
FL 60	52.0	PS-57 1	51.4	VB 316	55.6
FL 77	53.1	RT 06	54.5	VB 515	52.5
FLN 30	54.7	RT 09	55.1	VB 547	53.4
FLN 46	55.0	RT 106	55.0	VB 670	52.3
FSR 01	52.2	RT 06	51.0	VB 679	56.5
FSU 01	54.0	RVID 08	53.5	VB 892	53.8
FSU 07	53.7	RVID 12	56.2	VB 900	53.1
FSU 127	50.1	SCS 20	53.1	VB 902	51.4
FSU 13	54.7	SJ 02	53.6	VB 903	52.2
FSU 151	52.2	SM 02	50.7	**TSH 1188**	55.8
FSU 77	50.7	SM 04	52.5	**SIAL 169**	50.3
GM 33	55.0	SM 06	50.4	**SIC 23**	50.4

Group 5 (MLCT 05)

The clone trials were established in three locations between July 2005 and January 2006. Many clones of this group were also sent to the State of Pará to be tested in the district of Tomé-Açu. All clones were selected from the breeding populations generated in the CFC/ICCO/IPGRI project. Most of them are self-compatible genotypes with resistance to WB, desirable seed and pod characteristics and good yield. The clone trial established in the Corcovado farm is one year older than that of Jaci farm. The data analyses for the first 2 years showed variation among clones for yield and resistance. In these trials some clones are showing their yielding potential very early. In the Corcovado trial, several clones produced an accumulated mean superior to 8 kg of dry seed per plant during the period 2008-09, e.g. CP 47 (16.4 kg), CP 111 (8.8 kg), CP 115 (10.8 kg), CP 176 (17.4 kg), CP 196 (16.4 kg), CP 197 (11.1 kg), CP 230 (9.2 kg), CP 234 (15.6 kg), CP 237 (13.7 kg), CP 242 (10.2 kg), HB 7 (10.7 kg) and HB 15 (12.7 kg). The control varieties for resistance (TSH 1188) and susceptibility (SIAL 169 and SIC 23) to WB produced 7.9 kg, 7.2 kg and 6.0 kg, respectively. A peculiar characteristic of these clones is the presence of flower cushions with very little flowers and dispersed over the trunk and branches. Since they are self-compatible, almost all the flowers are fertilized and the fructification is significant. Also, in the Jaci farm, almost the same clones presented superior performance in respect to seed production and resistance to WB, but producing much more than the control varieties (SIAL 169 = 0.19 kg, SIC 23 = 0.35 kg and TSH 1188 = 0.40 kg). These superior clones were: CP 199 (1.14 kg), CP 223 (1.05 kg), CP 234 (1.49 kg), CP 155 (0.87 kg), CP 176 (0.88 kg), HB 7 (1.11 kg) and HB 15 (0.87 kg). In both clone trials HB 51 presented very low levels of resistance, similar to those of SIAL 169 and SIC 23. Clones CP 140, CP 236 and CP 242 presented moderate incidence of cushion brooms.

On-station breeding efforts

Clone selection

- **Local Clone Observation Plots (LCOPs)**

Advanced breeding populations were generated to support the programme of clone selection. All selected plants in these populations were cloned, multiplied and established in the field as observations plots. Each plot was represented by a row of 10 plants. The main interest of the LCOP is to maintain collections of on-farm and on-station cocoa selections under field and lab evaluation, enabling the identification of the more promising ones for the composition of new large-scale evaluation trials, or for the recommendation of planting materials at small scale. A total of approximately 425 clonal selections is being evaluated in the LCOP. Table 6 shows the overall mean for 275 CP selections and the maximum and minimum values observed for the following variables: number of healthy pod per plant (HP/Plant), percentage of healthy pods (HP%), percentage of pods attacked by *Phytophthora* (Ppr%), percentage of pods attacked by WB (WBP%), number of vegetative brooms per plant (VB/Plant) and number of cushion brooms per plant (CB/Plant). Although the range of variation is large, the proportion of CP selections with higher number of pods per plant, lower pod losses due to diseases and lower incidence of vegetative and cushion brooms is considerable.

Table 6. Overall mean and maximum and minimum values observed for 275 CP selections for pod and disease variables (for definition of variables see above)

	HP/Plant	HP (%)	Ppr (%)	WBP (%)	VB/Plant	CB/Plant
Mean	21.0	76.2	7.1	16.7	8.9	12.1
Maximum	133.5	95.9	32.1	49.3	98.1	206.8
Minimum	1.3	33.1	0.0	0.0	0.5	0.0

Population breeding

- **Population breeding and selection**

Eight breeding populations were generated between 2003 and 2005, constituted by 205 bi-parental crosses. Altogether 14 470 plants have been maintained and evaluated individually. Four of these populations were established in the field according to statistical block design with 3 to 4 replications and plots varying from 12 to 20 plants. The remaining populations were constituted by single observation plots of variable size. The main interest is for clone selection, but progeny selection will also be possible. Large variation was observed within and between the sub-populations, allowing the selection of a large number of clones with high productivity and resistance to diseases such as WB, Ppr and *Ceratocystis* wilt. This has enabled the selection of approximately 475 clones, identified as CP selections. Some of the newer populations were not submitted to clone selection yet, and some progenies present very good field performance in terms of productivity and resistance to diseases.

Validation of varieties in the Regional Variety Trial (RVT) in South America

A field trial was established with a randomized block design with 14 progenies, 4 replications and 12 plants per plot. Every participating country contributed to the RVT with some progenies. CEPLAC contributed with three progenies. The quarantine station for cocoa was used for reception of these progenies, and these materials were kept there for about 12 months.

The preliminary data of the RVT were analysed. The comparisons among progenies are quite complicated since they were planted in the field with different ages and also in different seasons. Therefore the RVT is regarded as a potential population that will provide germplasm for breeding purposes. The results of the analysis of variance (ANOVA) for variables related to pod and seed production and resistance to WB show that there are significant differences between progenies. The observed statistics for each progeny are presented in Table 7a for variables related to pod and seed production, and in Table 7b for variables related to resistance to WB. There is a large variation for all variables studied, which indicates ample possibilities for selection and breeding. This trial includes progenies involving parents with resistance to moniliasis, WB, Ppr and also to *Ceratocystis* wilt. Most of the progenies presented a high level of resistance to WB in the canopy, flower cushions and pods. In these aspects lies the importance of this germplasm.

Table 7a. Descriptive statistics of progenies for traits(1) related to pod and seed production in the Regional Variety Trial

Progeny	Frequency	#HP				#TP				DSW/P			
		Mean	Standard deviation	Minimum value	Maximum value	Mean	Standard deviation	Minimum value	Maximum value	Mean	Standard deviation	Minimum value	Maximum value
VB 184 X CP 50	48	3.44	7.45	0	30	4.31	7.86	0	30	135.83	292.13	0	1192
CP 40 X CP 87	41	2.37	5.73	0	27	3.68	7.40	0	33	98.93	255.79	0	1136
CP 52 X CCN 16	49	1.10	2.06	0	10	2.18	3.13	0	12	43.76	86.07	0	416
TSH 1188 X CCN 51	38	1.50	4.43	0	23	2.58	5.85	0	28	61.05	183.51	0	888
CCN 51 X CC 137	32	0.63	1.18	0	4	1.22	2.22	0	7	20.00	48.85	0	248
EET 183 X (P 7 X UF 273)	37	0.41	1.12	0	6	1.19	3.02	0	17	17.08	48.89	0	232
PA 169 X ICS 95	51	0.51	1.17	0	6	1.49	2.93	0	17	18.35	50.63	0	312
EET 233 X U 9	44	2.73	6.35	0	30	4.91	10.53	0	49	96.36	211.53	0	736
CCN 51 X TAP 12	56	0.63	1.53	0	7	1.21	2.16	0	8	24.86	62.45	0	288
TSH 973 X ICS 95	54	1.93	3.38	0	15	2.87	4.54	0	23	92.89	178.64	0	752
TRD 32 X PA 169	54	1.46	2.49	0	12	2.87	4.19	0	17	53.04	98.96	0	560
CCN 51 X EET 233	28	1.29	3.39	0	16	2.11	4.85	0	24	48.57	129.75	0	592
SCA 6 X ICS 1	52	4.79	7.27	0	26	6.52	9.19	0	39	200.15	296.75	0	1000
MIXT PERU	39	1.21	2.94	0	15	2.51	4.59	0	19	40.41	102.79	0	472

[1] Traits:
#HP = number of healthy pods
#TP = total number of pods
DSW/P = dry seed weight per plant

Table 7b. Descriptive statistics of progenies for traits[1] related to resistance to WB in the Regional Variety Trial

Progeny	Frequency	%WBAP				VB				CB			
		Mean	Standard deviation	Minimum value	Maximum value	Mean	Standard deviation	Minimum value	Maximum value	Mean	Standard deviation	Minimum value	Maximum value
VB 184 X CP 50	48	3.83	11.99	0	50	4.90	5.12	0	22	0.75	1.58	0	6
CP 40 X CP 87	41	11.84	25.99	0	100	5.07	5.90	0	24	1.05	1.73	0	7
CP 52 X CCN 16	49	9.62	24.34	0	100	3.84	4.03	0	17	0.71	1.87	0	10
TSH 1188 X CCN 51	38	4.68	11.78	0	37	5.50	5.21	0	18	0.71	1.47	0	6
CCN 51 X CC 137	32	3.70	11.11	0	33	7.09	7.19	0	29	0.56	1.37	0	6
EET 183 X (P 7 X UF 273)	37	18.14	32.14	0	100	8.03	7.71	0	28	0.73	2.10	0	11
PA 169 X ICS 95	51	18.89	33.55	0	100	7.41	6.59	0	29	0.92	2.12	0	13
EET 233 X U 9	44	7.05	15.33	0	50	6.07	6.31	0	24	1.16	2.18	0	7
CCN 51 X TAP 12	56	25.88	39.37	0	100	3.79	4.05	0	17	0.46	1.41	0	8
TSH 973 X ICS 95	54	7.89	22.56	0	100	5.87	4.91	0	23	1.41	2.25	0	8
TRD 32 X PA 169	54	13.76	28.20	0	100	11.11	11.84	0	60	1.54	3.19	0	19
CCN 51 X EET 233	28	13.18	23.46	0	66	6.64	7.39	0	30	0.96	2.10	0	8
SCA 6 X ICS 1	52	7.44	19.12	0	100	4.12	5.14	0	22	2.98	5.74	0	30
MIXT PERU	39	16.52	28.52	0	100	5.79	6.09	0	26	1.10	2.72	0	14

[1] Traits:
%WBAP = percentage of pods attacked by WB
VB = number of vegetative brooms
CB = number of cushion brooms

Resistance testing studies

Assessing resistance on cocoa seedlings

- **Droplet and spray inoculation of seedlings from open-pollinated pods of two genotypes**

This experiment was proposed to compare and validate the two methods to be used as early selection in pre-breeding or in accelerated hybrid clone selection programmes for WB resistance using open-pollinated seedlings of two genotypes, one resistant and one susceptible.

Five pods resulting from open-pollinations were harvested from five trees of the ICS 1 and MO 20 clones. Seeds of all pods were mixed and sown in garden bags filled with top soil, covered with a horticultural potting mixture. One hundred and twelve seedlings of each clone were inoculated with each of the following methods: (i) spray method: 1 ml of a calibrated basidiospore suspension delivered to the active terminal buds of each plant; (ii) agar-droplet method: a 30 µl drop of a calibrated basidiospore suspension in 0.3% agar was placed on the active terminal buds according to the method used by Surujdeo-Maharaj et al. (2003).

The inoculum concentration used was 1.0×10^5 basidiospores/ml. Evaluations were done 60 days after inoculation. The symptoms observed were swellings (SWE), terminal broom (TB), axillary broom (AB) and cotiledonary broom (CB). The diameter of the terminal broom was measured when this symptom was present. Two variables were analysed: SINT (comprising all kind of symptoms) and DVT (terminal broom diameter).

After the first inoculation, 49.1% of the ICS 1 plants and 37.5% of the MO 20 plants inoculated by the spray method were infected, while from the plants inoculated by the agar-droplet method 68.8% of the ICS 1 and 33.9% of the MO 20 plants were infected. There was no statistic difference between methods but clones had different reactions when inoculated by both methods (Table 8). After the evaluation plants were pruned and organically fertilized.

Table 8. Numbers of inoculated and infected plants of ICS 1 and MO 20, 60 days after inoculation through both methods, at three different times (R1, R2, R3 = replications)

Clones	No. of inoculated/infected plants					
	Spray method			Agar-droplet method		
	R1	R2	R3	R1	R2	R3
ICS 1	112/55a	82/13	73/14	112/77a	81/14	68/10
MO 20	112/42b	95/13	86/12	112/38b	95/13	88/10

The second inoculation of the same ICS 1 and MO 20 plants was accomplished in December 2007. The first symptoms in ICS 1 plants were observed 3 weeks after the inoculation and plants of MO 20 expressed symptoms only during the fourth week after inoculation regardless of the inoculation method used. This was common for all the three inoculation times. Sixty days after inoculation, 15.9% of the ICS 1 and 13.7% of the MO 20 plants showed disease symptoms when inoculated by the spray method. When the agar-droplet method was used for inoculation, 17.3% of the ICS 1 plants showed symptoms as compared to 13.6% of the MO 20 plants (Table 8). There were no significant differences between the reactions of ICS 1 and MO 20 plants to WB infection for both inoculation methods and the methods did not differ from one another. Plants with and without symptoms were pruned and organically fertilized again.

The third inoculation of the plants of ICS 1 and MO 20 was made in July 2008. Sixty days after inoculation, 19.2% of the ICS 1 and 14% of the MO 20 plants showed WB symptoms when inoculated by the spray method. When the agar-droplet method was used for inoculation, 17.6% of the ICS 1 plants showed symptoms as compared to 11.4% of the MO 20 plants. There were no significant differences between the reactions of ICS 1 and MO 20 plants to WB infection for both inoculation methods and neither between the inoculation methods.

Thirty-tree plants of this experiment (22 of ICS 1 and 11 of MO 20) that showed symptoms more than once after the three previous inoculations were re-inoculated with the agar-droplet method using the inoculum concentration of 3×10^5 basidiospores/ml, i.e. three times higher than in the previous inoculation (1×10^5 basidiospores/ml). The evaluation of these plants was done daily. First symptoms appeared 20 days after inoculation for ICS 1 plants and 25 days after inoculation for MO 20. Sixty days after inoculation, 77.3% plants of ICS 1 and 72.7% of MO 20 were infected. Four inoculated plants of ICS 1 and two of MO 20 died as an effect of WB symptoms. There was no difference in clone reaction for both pathometric variables evaluated (Table 9).

Table 9. Number and percentage of infected plants of ICS 1 and MO 20, approximately 2 years old, inoculated through the agar-droplet method using 3 x 10^5 basidiospores/ml

Clones	No. of inoculated plants	No. of infected plants	No. of dead plants	% of infected plants	% of dead plants
ICS 1	22	17	4	77.3	18.2
MO 20	11	8	2	72.7	18.2

The same procedure was followed for 282 plants (163 plants of MO 20 and 119 of ICS 1) of the same experiment that either never showed symptoms or were infected only once (first inoculation) after being inoculated three times with the inoculum concentration of 10^5 basidiospores/ml. The fourth inoculation was done to find out whether, even with three times more inoculum (3 x 10^5 basidiospores/ml), the plants would still show a resistant reaction to *Moniliophthora perniciosa* infection. However, 60 days after inoculation, 73% of the ICS 1 plants and 65% of the MO 20 showed symptoms, and 18.5% of the ICS 1 plants and 7.4% of MO 20 died as an effect of WB symptoms. There was no difference in clones' responses to inoculation according to ANOVA (Table 10).

Table 10. Number and percentage of infected plants of ICS 1 and MO 20, approximately 2 years old, inoculated though the agar-droplet method using 3 x 10^5 basidiospores/ml

Clones	No. of inoculated plants	No. of infected plants	No. of dead plants	% of infected plants	% of dead plants
ICS 1	119	87	22	73	18.5
MO 20	163	106	12	65	7.4

- **Droplet inoculation of seedlings (open-pollinated and hand-pollinated pods) and clonal plants of six genotypes**

The objective was to compare and validate advanced WB resistance testing methods in several countries to be used as early selection in pre-breeding or in accelerated hybrid clone selection programmes. Three different experiments were carried out.

Experiment 1

Seedlings of six clones (SCA 6, MO 20, PA 150, LCTEEN 46, AMAZ 15-15 and NA 33) were obtained from a mix of seeds of 10 open-pollinated pods of each clone. One hundred and twelve seedlings of each clone were inoculated with each of the spray and agar-droplet inoculation methods, using an inoculum concentration of 1 x 10^5 basidiospores/ml. The inoculations were done in July 2007 and the symptoms were recorded since the first week after the inoculation to observe the exact day of appearance of the first symptom. However, since all six clones were resistant to WB, they expressed only a few disease symptoms, the percentage of infection varying from 3% (SCA 6) to 8% (NA 33) as expected. The test was not considered successful.

Experiment 2

Five pods resulting from manually-pollinated flowers of seven clones (SCA 6, MO 20, AMAZ 15-15, LCTEEN 46, ICS 1, Playa Alta 2 and EET 59) were harvested for each clone. Twenty seeds per pod were mixed and sown in garden bags filled with top soil, covered with a horticultural potting mixture. Thirty seedlings of each clone were inoculated in October 2008 through the agar-droplet (T2) and spray (T1) methods. The inoculum concentration used was 1 x 10^5 basidiospores/ml. The symptoms were recorded since the first week after the inoculation to observe the exact day of appearance of the first symptom and the final evaluation was done 60 days after inoculation. For data analysis the values of SINT (all kind of symptoms) were transformed to arcsine of the square root.

Data analysis showed that the agar-droplet method was more effective than the spray method. However, there was no difference among clones for both methods (Table 11). Plants with and without symptoms were pruned and organically fertilized.

Experiment 3

A second inoculation was done six months later using only the agar-droplet method and 60 plants for each clone with the exception of clones EET 59 and Playa Alta 2. The inoculum concentration used was 3 x 10^5 basidiospores/ml. There were differences among clones for WB infection and the percentages of infected plants varied from 10.0% (MO 20) to 44.1% (EET 59). MO 20 and SCA 6 were resistant although not differing from LCTEEN 46, AMAZ 15-15, ICS 1 and Playa Alta 2 (Table 11).

Table 11. Percentage of infected plants of seven cocoa clones inoculated with the spray and agar-droplet methods. Data were recorded 60 days after inoculation

Clones	Percentage of infected plants			
	Spray method	Agar-droplet method		
	R1	R1	R2	
MO 20	0.0	13.3	10.0	b
SCA 6	0.0	26.6	11.7	b
EET 59	10.0	23.3	44.1	a
LCTEEN 46	13.3	26.6	20.0	ab
AMAZ 15-15	13.3	3.3	16.7	ab
ICS 1	13.3	20.0	18.6	ab
Playa Alta 2	10.0	43.3	34.5	ab
CV	**51.9**	**24.0**		

R1, R2 = replications
R2: numbers followed by the same letter were not different according to Tukey's Test ($P \geq 0.05$).
CV = coefficient of variation

Assessing resistance of cocoa pods

- **Development of a method for evaluation of pod resistance to WB**

Attacks of *M. perniciosa* on pods can lead to several kinds of symptoms depending on the age of the cherelles when infection occurs. The incubation period before the first symptom appears (several weeks) does not permit the development of a test using detached pods. Experiments were carried out with the objective of testing a methodology to assess the response of cocoa pods to WB.

Experiment 1

Four clones, two of them with supposed pod resistance to WB (SCA 6 and MO 20) and two susceptible ones (ICS 1 and Playa Alta 2) were chosen. Ten hand-pollinated cherelles at ages of 40, 60 and 80 days were inoculated for each inoculum concentration (20 cherelles per clone). The inoculations were done by: 1) spraying approximately 0.5 ml of the inoculum suspension on each cherelle (avoiding run-off of inoculum) from a distance of 15 cm; 2) adding a Whatman's paper disc #2 dipped into the inoculum suspension at each side of the cherelles. Three basidiospore concentrations were used (0 as control, 5 x 10^5 basidiospores/ml and 1 x 10^6 basidiospores/ml). All pods were maintained inside polyethylene bags during 30 days after inoculation. Observations were done weekly and the final evaluation was done 90 days after inoculation.

Data analysis showed significant effects of the age of the pods, genotype tested and inoculum concentration. There were no effects of inoculation method or of the interactions. The inoculum concentration effect did not differ for the two concentrations used. Only two control pods of ICS 1 were infected (infection must have occurred before inoculation time); all the others were healthy. No differences were observed for the two inoculum concentrations (5×10^5 and 1×10^6 basidiospores/ml). More infection was recorded on 40-days-old pods of ICS 1 and Playa Alta 2 clones. There was no difference between the other two ages (Table 12). SCA 6 and MO 20 were different from ICS 1 and Playa Alta 2 in relation to pods reaction to WB infection, confirming field results.

Table 12. Number of inoculated and infected pods of three ages of each of four clones tested with two inoculum concentrations of basidiospore suspensions of *Moniliophthora perniciosa*

Clones	Number of inoculated pods / number of infected pods		
	40 days old	**60 days old**	**80 days old**
ICS 1	20 / 17	20 / 13	20 / 08
Playa Alta 2	20 / 18	20 / 10	20 / 07
MO 20	20 / 06	20 / 02	20 / 02
SCA 6	20 / 05	20 / 02	20 / 01

Experiment 2

Five clones, three of them supposed to be resistant to WB (MO 20, LCTEEN 46 and SCA 6) and two susceptible (ICS 1 and Playa Alta 2) were hand-pollinated to obtain 40-days-old pods. The pollinations were done with a standard male pollinator. Pollinations and inoculations were done at three different times (April 2008/June 2008; November 2008/January 2009; December 2008/February 2009). However, due to weather conditions, few flowers were available for pollination and the number of pods obtained was not sufficient to carry out the experiment using the four inoculum concentrations proposed. Therefore only the concentration of 1×10^5 basidiospores/ml was used. Symptoms observations were done weekly until 60 days after inoculation. Pods of MO 20 were inoculated only once (5 pods) and none were infected. The numbers of pods inoculated and infected per inoculation time are shown in Table 13. None of the control pods were infected. In the same Table 13, it is observed that the percentage of pods infected in all three inoculations varied from 8.7% (SCA 6) to 73.1% (Playa Alta 2). For data analysis the values of pods infected were transformed to arcsine of the square root. Only SCA 6 was different from the other clones that had similar susceptible reaction to WB, as was expected.

Table 13. Number of pods inoculated, infected and control, and percentage of pods infected by *M. perniciosa* per clone by using an inoculum concentration of 1 x 10^5 basidiospores/ml

Clones	No. of inoculated pods				No. of infected pods				No. of control pods				Ratio A/B	% of infected pods	
	R1	R2	R3	Total (A)	R1	R2	R3	Total (B)	R1	R2	R3	Total			
MO 20	5	0	0	5	0	0	0	0	3	0	0	3	5/0	0.0	
SCA 6	10	10	3	23	1	0	1	2	5	3	3	11	23/2	8.7	b
MAN 15-2	10	10	10	30	7	4	4	15	5	5	5	15	30/15	50.0	a
ICS 1	10	10	10	30	8	5	4	17	5	5	3	13	30/17	56.7	a
Playa Alta 2	10	10	6	26	7	6	6	19	5	5	3	13	26/19	73.1	a

Numbers followed by the same letter were not different according to Tukey's Test ($P \geq 0.05$).
Data transformed to root square of the arcsine of the variable pods infected.
R1, R2, R3: replications

Experiment 3

Pods of 15 cocoa accessions were tested for pod resistance using the pod inoculation test of point disc inoculation. The method was able to separate resistant and susceptible materials. Some resistant materials under field conditions were also resistant to inoculation (Figure 4).

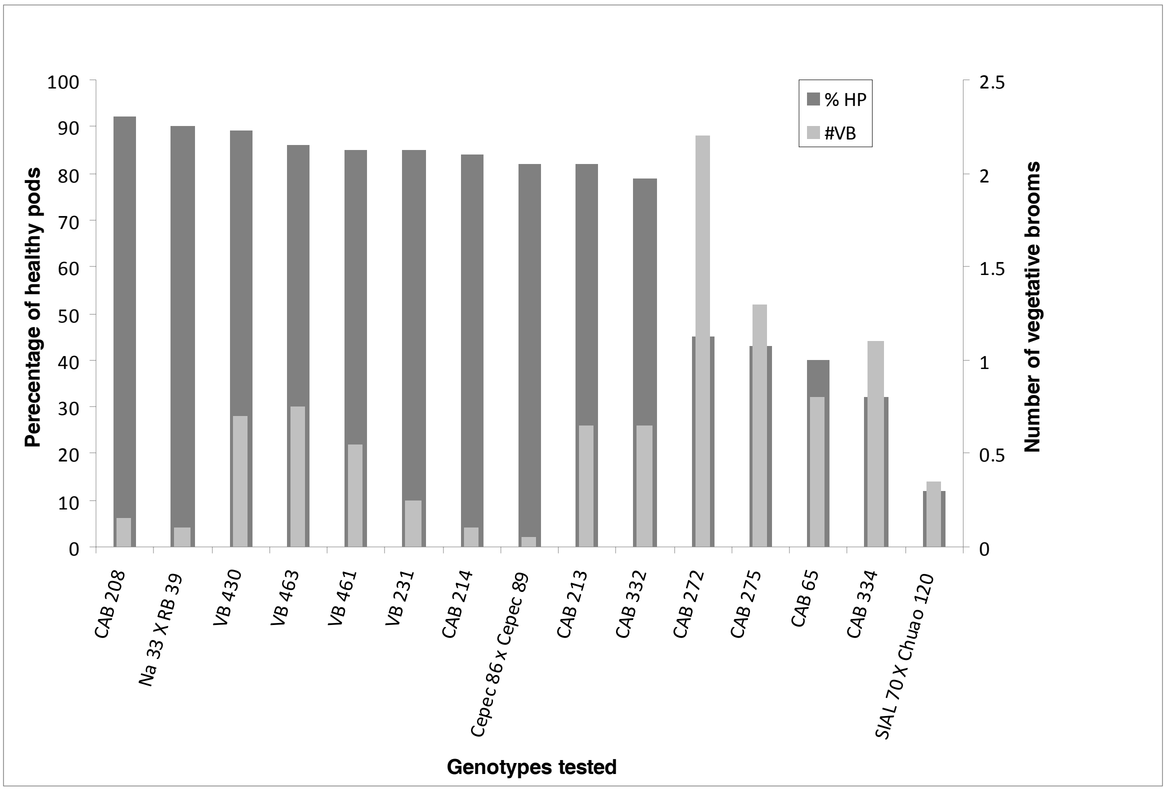

Figure 4. Percentage of healthy pods and number of vegetative brooms observed in the field experiment for the 15 genotypes tested.

Lessons learned

Once working with clones, breeding populations need to be generated in order to promote association of desirable traits, to increase the frequency of favourable alleles especially related to yield, seed quality and resistance to disease. There is no need to evaluate the populations for more than five years. Besides that, the plants can be planted in smaller spacing, such as 3 m x 1.5 m, and the yield potential of individual plants can be measured by concentrating the counting of pods over only 2 or 3 months in the main and in the secondary harvesting seasons.

The idea of international variety trials was valid, but they involved complex procedures, which became serious limiting factors. For instance, the impossibility of producing the same set of progenies at the same season and of respecting the best planting times in the participating countries did not allow the comparisons between progenies. Besides, quarantine services are required. In addition, there was a case of packing violation, resulting in the mixing of seeds of different progenies. The efforts dedicated to the production of the progenies are great and there is a high risk of losing them.

Impact of the CFC/ICCO/Bioversity project

This project had an important role not only for the cocoa producers of Bahia, but also for producers of other States of the Union and all other producer countries. Even the new, non-traditional cocoa areas are benefiting from it. A large number of clones could be tested over different environments, allowing them to be adequately evaluated. Twenty clones here tested are already being recommended to the cocoa producers for planting. These are high-yielding and resistant to WB and Ppr. All these clones are also self-compatible and the bean quality is within acceptable values. Many practices on clone management were learned from the clone trials, FSOP and LCOP, which are useful not only for Brazilians, but also for farmers from other countries who are using clones. This will help the producers to rehabilitate their plantations through the correct use of planting material, which will guarantee the expected success.

During the project adequate training was provided on participatory research to various cocoa professionals from Brazil and other participating countries from Central and South America and the Caribbean region, through intensive theoretical and practical courses in Brazil and Ecuador. The training enabled each country to plan its participatory activities for all specific situations, including those under the CFC/ICCO/Bioversity project. The training on participatory research also strengthened the link between researchers, extension agents and producers, resulting in adequate diagnosis of problems and selection of interesting planting materials, thus increasing the varietal options. It was possible to identify some preferences for specific types of cocoa varieties, enabling adjustments on the selection criteria. Some farm selections were validated by CEPLAC, e.g. PS 1319, CA 1.4 and RVID 08.

Other good farm selections are being observed by CEPLAC, especially through the network of clone evaluation established by the project. This way, many other selections may be validated and released in a short time.

The population breeding programme is quite large and advanced, which allows selecting more productive and resistant genotypes with a broad genetic base. The impact of the establishment of these populations will be perceived not only now, but also many years from now, through the selection in populations arising from these original ones established by the project.

Indirectly the project enabled the participating countries to have access to very important cocoa germplasm through the Regional Variety Trial (RVT) and CFC Collection maintained at the University of Reading, UK. The breeders can now use these facilities to breed for resistance to exotic diseases and have access to better germplasm from other countries.

Under the supervision of Dr Edna Dora, two students developed MSc dissertations on very important subjects: a) Characterization of the resistance gradient of cocoa genotypes (F2 SCA 6 x ICS 1) to black pod disease using phytopathometric, genetic and molecular approaches (Santos 2009); and b) Pathogenic variability of *M. perniciosa* from three agro-ecological zones of the cocoa-growing region of Bahia (da Silva 2009). In addition, two other agronomists hired by the project received very good training on cocoa breeding and cocoa pathology, and the technicians involved in this project participated significantly in the dissemination of all technologies generated to the producers.

An important lesson related to the method of propagation employed could be drawn from the clone trials. In the farms, clones are usually grafted on basal chupons of adult cocoa trees. The same procedure was used for the establishment of the clone trials. The use of this propagation procedure resulted in serious problems during the development of the clone trials, such as increasing variability within clones with age, difficult handling of the plants, excessive mortality, decrease in production and malformation of the root system. This suggested that CEPLAC's recommendations to the producers may need to be reviewed in relation to the more appropriate methods for propagating clones. It is suggested to use nursery-grafted or budded seedlings or, even, to plant rootstock seedlings previously in the field to be grafted after 10 months approximately. Thus, by using a standard procedure it is possible to increase the uniformity within clones due to easy handling of the plants, reduction of mortality, etc. Consequently, the producers will certainly achieve a higher productivity.

During the years of the project implementation at CEPEC it was possible to establish a methodology to assess cocoa genotypes for resistance to *Ceratocystis* wilt (CW), a disease that became severe against some of the selected clones or varieties distributed with resistance to WB. Hundreds of cocoa genotypes were screened; some showed resistance to CW and are now being been used by farmers as cocoa rootstock.

It was shown that the agar-droplet and the spray method are both equally efficient to test resistance of cocoa genetic materials to *M. perniciosa*. The choice of each method will depend on the number of genotypes and plants/genotype to be tested. The agar-droplet method is recommended when less than 600 plants must be inoculated at a given time and the spray method for a larger number of plants. Two students, one biologist and one agronomist obtained their MSc degrees doing complementary research applicable to improve the cocoa breeding programme for resistance to both black pod and witches' broom diseases. It was demonstrated that there was variability in the disease response of cocoa genotypes to inocula of *M. perniciosa* from different agro-ecological zones, the inoculum from Ipiau agrosystem (and county) being the most aggressive. These findings have a very practical importance and show the need of continuing the survey of resistant materials to be tested against the inocula from specific locations where they will be grown. The possibility of using a mix of inocula of *M. perniciosa* from different regions to select for resistance must be further investigated.

Research needs

Participative genetic improvement

Potentially interesting populations could be produced by the cocoa breeders and distributed to different producers for planting and observation in their own farms. They will evaluate the field performance of these progenies in respect to productivity and disease resistance, as well as the best selected plants according to their preference and criteria.

Innovations on cocoa breeding

A new way of testing cocoa progenies can be proposed, in which the progenies are planted at high density and the best plants would be selected within and between progenies during the first 2 or 3 years of bearing. All unselected plants would be discarded and each selected plant would be cloned and replicated 4 to 5 times. This could be a dynamic process, where large number of progenies would be tested in small areas, and only the selected plants would be preserved for further evaluation. Many of these selected plants that continue expressing attractive yield, seed quality and resistance after 4 years of observation would be delivered to the producers for planting. They will decide which ones they like most and intensify their multiplication. Such a method deserves to be tested in cocoa. Still, based on previous experiences, we could reduce the frequency of field measurements for most of the traits without any major prejudice to the reliability of the comparison between genotypes. Molecular biology and biotechnology tools would be useful to carry out marker-assisted selection for resistance and multiplication of genotypes.

Plant management

One of the main causes of the low productivity of the cocoa plantations is the plant formation. When it is not done adequately right from the establishment of the plantation, the competition among plants becomes very intense with age, and consequently the production drops drastically and the mortality of plants, as well as the frequency of unproductive plants, increase.

Spacing x varieties interaction and/or rootstock x clone interaction

Both types of interactions need to be much more studied. Whenever working with clones it is necessary to define the best spacing and rootstocks for each specific clone. Using for instance very vigorous seedlings as rootstocks may have a very positive influence on the growth rate of the clones. If more vigorous clones are grafted on these rootstocks, the final plants will certainly be much bigger, making the handling more difficult, thus increasing the competition among plants, especially for inadequate plant spacings. Clones with different growth rates require specific combinations of rootstocks and plant spacing. If someone decides to establish a plantation at high density and in monoclonal blocks, for instance, it is necessary at least to investigate rootstocks that may reduce the growth rate of vigorous clones or to identify less vigorous clones that facilitate handling. Even when working with progenies the questions of plant spacing and adequate handling of plants are fundamental for the success of the enterprise. What would be the more appropriate canopy shape of the clones in the various planting densities?

On the other hand, it is necessary to decide how clones should be propagated in order to facilitate the handling. If fan branches (plagiotropic branches) are used, more pruning will be required to adapt the canopy shape and size within the planting spacing planned. If orthotropic branches are used, a seedling type of canopy will be obtained. The same results can be achieved via somatic embryogenesis.

In this proposal, it is strongly recommended to elaborate well-illustrated publications directly addressed to cocoa producers and field cocoa workers, in particular using an adequate and simple language. This kind of information seems never to reach the target audience. We have been observing that most of the important information that determines the success of a plantation reaches the right persons with difficulty and when it does, the quality of the information is not as expected. Therefore most cocoa plantations show chronic problems limiting the production and longevity of plants.

Establishment of demonstration plots of cocoa cultivation

In order for the results of the project to fully reach the farmers, it is proposed to establish demonstration plots in farmer fields, using the best clones developed by the CFC/ICCO/Bioversity project and the management technologies developed in the proposed project. These plots could be areas of 3 ha per farm, using monoclonal blocks. These areas would be strategically chosen in the cocoa region to be visited by a large number of producers.

Development of agroforestry systems with cocoa

The sustainability of cocoa plantations has been questioned everywhere. Many of the cocoa plantations have adopted as permanent shade trees species with little or no economic value. Besides, the great majority of the plantations are excessively or inadequately shaded. In such conditions any cocoa plantation becomes unproductive. It is necessary to adapt the canopy of these shade trees, allowing more sunlight to come through. Various forest species could be used as shade trees in substitution of the non-economic species that are currently being used. Besides of the choice of shade species, it is necessary to give them a good formation pruning to prevent the canopies of these trees from becoming so big that they eventually prevent sunlight from coming through. These species need to be planted with adequate plant spacing that favours both crops. In Brazil, some cocoa plantations in the process of rehabilitation are substituting the shade trees by clones of rubber trees with better canopy architecture.

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Farmer participatory and collaborative approaches to cocoa breeding in Cameroon

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Introduction

Cocoa production constraints

Several constraints exist to cocoa production in Cameroon:

- **Agricultural and agronomical constraints**

Age of the cocoa trees in cocoa farms
Most of the cocoa farms in Cameroon are older than 50 years and their productivity is poor due to a drastic reduction in density and in yield of surviving trees.

Agricultural practices
Most cocoa farms are usually poorly managed in terms of:
- planting: planting under excessive shade, in absence of proper spatial design for repartition between cocoa trees and other perennial crops and forest trees, inducing strong competition effects;
- plot management: absence of pruning and of proper insecticide treatment.

- **Pests and diseases**

Dieback
Until now, it is assumed that the "dieback" symptoms, resulting in death of adult trees, from a young age (it starts 2 years after planting), are caused by a fungal attack in which *Lasiodiplodia* is involved. Anyhow, the causal or opportunistic role of this fungus is not clear as yet. This disease is particularly problematic since no method has been found to control it and it can seriously jeopardize the survival of cocoa plots, since more than 30% of the cocoa trees have died from this disease in some on-farm trial plots set up in 2006.

Mirids
Cocoa farmers rarely apply the right insecticide at the right moments and do not treat at all in most of the cases. Contrarily to black pod, they do not clearly perceive the damages caused by mirids and they do not appreciate the effects of insecticides. There are some punctual governmental actions in order to apply insecticides in some regions but these actions are usually not consistent.

Black pod disease
Despite the seriousness of this disease, this is the least problematic of the three phytosanitary problems in Cameroon, since farmers are accustomed to apply fungicide and can afford it now, thanks to the high price paid for their cocoa.

- **Access to improved varieties**

It is estimated that around 10% of the demand for improved varieties is fulfilled by the extension units in charge. This situation can be explained by the poor agronomical condition of the existing seed gardens, which are usually characterized by a low cocoa density, due to ageing of these plots. Until a recent past, the demand for improved varieties was very low, so that these plots were more than sufficient to fulfil it. But this demand has strongly increased since the last 3 years and the extension units are now facing a dramatically insufficient pod production.

Cocoa breeding objectives

The first objective of cocoa breeding in Cameroon is to select varieties adapted to the different local conditions prevailing in the country, through the assessment of trial plots set up in different regions (Centre, South and Southwest Cameroon) and in different conditions (forest, fallow, savannah). Adaptation means proper establishment ability followed by a high level of yield. Among the most promising progenies and clones, those showing the lowest level of susceptibility to black pod disease, to mirids and to dieback, will be selected for future release to farmers, for new planting (seedlings) or regeneration of old cocoa plots through field grafting (budwood).

The second objective is to find alternative solutions for farmers who do not have access to improved varieties through the official channel. These solutions are as follows:

- Setting up small-scale seed gardens managed by farmers and farmers' organizations for release of improved varieties to neighbouring farmers,
- Assessing the value of cocoa seedlings issued from farmers' fields with or without selection by farmers.

Current status of cocoa varieties

Unselected varieties

To capture the range of genetic diversity and the proportion of selected and unselected varieties in farmers' field, 400 farmers' accessions were fingerprinted along with 130 breeders selections used as reference. The results suggest the predominating presence of Lower Amazon genes (unselected) in the Cameroon farm accessions and a high level of admixture, with apparent presence of genes of more than three cocoa genetic groups in most accessions.

Most of the cocoa trees cultivated by farmers are issued from seeds collected in existing fields. Old cocoa farms were originally planted with the traditional variety, composed of Amelonado type trees ("German Cocoa"), but are now mainly composed of mixtures of German Cocoa and trees issued from fields set up with "hybrids" (selected varieties) or progenies issued from hybrids (open-pollination). Indeed, most farmers prefer to replace the dead cocoa trees and to extend their plots using progenies issued from selected varieties.

Recommended varieties

The following progenies are now being released by the extension units (Société d'exploitation du cacao (SODECAO) and Programme Semencier Cacao Café (PSCC)):

ICS 46 x T 60/887	T 79/501 x SNK 13 (+ reciprocal)
IMC 67 x SNK 64	T 79/501 x SNK 413
IMC 67 x SNK 109 (+ reciprocal)	T 79/501 x SNK 109 (+ reciprocal)
SNK 16 x SCA 12 (+ reciprocal)	UPA 134 x ICS 40
SNK 16 x T 60/1174	UPA 143 x SNK 64
SNK 16 x T 79/467	UPA 143 x SNK 413
T 79/501 x SNK 16	UPA 143 x T 79/501 (+ reciprocal)

Multiplication of recommended varieties

The recommended varieties are issued from bi-clonal seed gardens established in the 1970s in four areas of Cameroon (South, Southwest, East and Centre).

The seed garden in the Southwest is managed by PSCC and pods are directly released to farmers. The three other seed gardens are managed by SODECAO and seedlings are released to farmers after having been raised in nurseries localized in different areas and managed by SODECAO or private contractors.

Because of the poor condition of the seed gardens, it is estimated that only around 10% of the demand is fulfilled.

Recently, three small-scale seed gardens (0.25 ha) were set up by the Institut de Recherche Agricole pour le Développement (IRAD) in old cocoa plots, through on-field old cocoa grafting technique. The owners of these seed gardens will be trained to apply hand-pollination technique and will be in charge of the management of these plots. These seed gardens were set up with around 20 parental clones (all selected for tolerance to black pod disease) in each plot, and are designed to provide local farmers with mixtures of progenies cumulating several sources of tolerance to black pod disease.

Farmer participatory selection activities

Farm surveys

About 120 farms were surveyed in the different cocoa-growing areas of the country including the South, Southwest, Centre and East regions. In addition to these farms in the same regions, 400 farmers were interviewed on the origin and the types of cocoa varieties found in their plantations.

Farm selections

A total number of 271 cocoa trees were selected for yield and tolerance to black pod disease in cocoa farms from different regions of Cameroon (South, Southwest and Centre). Among these, 253 were cloned by grafting and are now in germplasm collection plots in IRAD research stations of Nkolbisson and Barombi-kang, while 18 have not been cloned as yet. All these clones and few open-pollinated progenies selected on-farm were assessed for their resistance to black pod disease, using the leaf inoculation test. On-farm trials are under way to assess 18 of these farm selections as clones and 55 as progenies issued from open-pollination.

On-farm trials

A total number of 109 trial plots have been established between 2006 and 2009, among which 74 are still under assessment. These trials were set up in two areas of Cameroon: the region of Yaoundé and the region of Bafia (north of Yaoundé).

Most of the plots (53) were set up on fallows, 15 of them on savannah and 6 on forest land.

Cocoa trees are intercropped with fruit trees (citrus, safou and avocado trees) in 50 of the trial plots, with oil palm trees in 15 trial plots, coconut trees in 6 trial plots while only cocoa trees were planted in 3 of the trial plots.

The following material is currently being compared in on-farm trial plots: 25 clones, 16 commercially released varieties, 54 progenies issued from controlled crosses at IRAD and 10 different types of progenies issued from farmers' fields (German Cocoa, progenies issued from unselected trees in farmers' fields planted with hybrids, progenies issued from open-pollination of trees selected in farmers' fields).

The clones and progenies in the plots set up in 2006 are currently assessed for yield and tolerance to mirids and to dieback and will be assessed for additional traits, such as resistance to black pod disease (pod inoculation test), number of beans per pod, mean weight of one bean and cocoa quality.

The varieties in the trial plots set up more recently are assessed for establishment ability, vigour and precocity (flowering and fruiting).

On-station breeding efforts

Clone selection

Twenty-three clones are currently assessed in a clone trial on IRAD research station of Nkolbisson. Nineteen of these clones are issued from trees selected in breeding trials for their level of yield and tolerance to black pod disease; two others are issued from trees selected on local farms for their level of yield. The trees are currently assessed for their level of yield, tolerance to mirids and to dieback, number of beans per pod and mean weight of one bean. They were also assessed for their level of tolerance to black pod using leaf and pod inoculation tests but the absence of *Phytophthora* on this plot since its establishment makes it impossible to assess tolerance to this disease in the field.

Hybrid selection

Three progeny trials are currently under assessment. The first one is the Regional Variety Trial (RVT), set up in 2005 on the research station of Barombi-kang (Southwest), in which 22 progenies are currently assessed (9 from Cameroon, 6 from Ghana, 5 from Côte d'Ivoire and 2 from Nigeria). The results so far are as follows:

The highest-yielding progenies are issued from the following crosses:

GU 144/C x EQ X3338 (Ghana)
SNK 614 x SCA 24 (Cameroon)
GU 144/C x NA 33 (Ghana)
IFC 303 x PA 121 (Côte d'Ivoire)
SNK 625 x NA 33 (Cameroon)

The lowest-yielding ones are issued from:

PA 4 x Pound 7 (Côte d'Ivoire)
T 85/799 x PA 120 (Nigeria)
SNK 614 x SNK 608 (Cameroon)

The results from the leaf inoculation test are as follows:

Progenies with the highest level of resistance to *Phytophthora* are issued from:
PA 107 x SNK 614 (Cameroon),
(Pound 7 x PA 150) x IMC 47 (Nigeria)
PA 107 x ICS 40 (Cameroon)
UPA 134 x SNK 64 (Cameroon)
SNK 614 x SCA 24 (Cameroon).

The most susceptible progenies are issued from:
T 85/185 x T 60/78 and its reciprocal (Ghana)
T 63/967 x T 17/524 (Ghana)
PA 13 x P 19 (Côte d'Ivoire)
IFC 303 x PA 121 (Côte d'Ivoire)
SNK 12 x PA 150 (Côte d'Ivoire).

The two other trial plots were set up in 2009 on the research station of Nkoemvone (in the South). Their main objective is the assessment for field tolerance to black pod disease of progenies currently assessed in on-farm trials. Thirty-one progenies are currently assessed in these two trial plots.

Lessons learned

The participatory breeding activities allowed us to establish a strong partnership with local cocoa farmers. This methodology showed both advantages and constraints.

Advantages

- Possibility of setting up large numbers of trial plots in different conditions, in terms of environment, but also in terms of field management. These variable conditions are very useful to assess the adaptability of varieties under evaluation.
- Most of the farmers were able to set up their trial plots according to the spatial (position of cocoa trees and fruit trees) and statistical (position of the progenies) designs, to make yearly recording of the dead cocoa trees and to replace them with trees from the same varieties. The seven most involved farmers have been able to record regularly the number of harvested pods on every cocoa tree in their trial plots.
- Three of the farmers were enthusiastic about enriching our collaboration by taking part in agronomical experimentation applied to their old cocoa plot regeneration, through chupon grafting following cutting down of old cocoa trees. These techniques are used for the setting up of on-farm clone trials, germplasm collection plots and seed gardens.
- These activities allowed us to train the involved farmers to agronomical practices applied to cocoa (proper planting, pruning) and to other crops (fruit tree grafting, plantain vegetative multiplication).

Constraints

- Farmers' enthusiasm declines with time; we need to be prepared to abandon some of the on-farm trials.
- Need to adapt to farmers' conditions, in terms of land (sometimes need to modify the spatial design just before planting) and field management (farmers perform fungicide treatments, making it difficult to assess tolerance to black pod, and most of the farmers prepare their fields for planting at the last moment, resulting in a late planting period).

- Need to cope with farmers' legitimate eagerness to get high productivity on their plots, which forces to find a balance between scientific and economical interests. For example, some of the poorly establishing varieties were replaced one or two years after planting, even if they are important from a scientific point of view (e.g. German Cocoa, which is a very useful control, but which is not adapted to our field management practices).
- Need to keep a regular contact with farmers, resulting in frequent trips, and consequently a high cost.

Conclusions

Impact of the CFC/ICCO/Bioversity project

- **Impact on IRAD**

This project allowed a dramatic increase of breeding activities, especially with the Participatory Breeding Programme. These activities would never have been possible without the purchase of the vehicle with the funds of the project. This programme has also resulted in a strong collaboration between breeders and agronomists. The strong collaboration with the farmers involved in the project has resulted in a network of 74 breeding trial plots in six different geographical sites. In addition, some of these on-farm trial plots are now also used by plant pathologists and entomologists for epidemiology studies.

- **Impact on cocoa farmers**

Around 100 cocoa farmers have been involved in the project, which has given them an opportunity to have access to improved varieties of cocoa, citrus and oil palm trees as well as to technical training (nursery, planting, plot management, grafting, vegetative multiplication) for cocoa and other crops (oil palm trees, fruit trees, plantain). The farmers are also required to score all the input and output from their plots in order to enable them to evaluate the profitability of their plots, which is expected to be much higher (at least four times) than that observed on traditional cocoa plots.

- **Social and environmental impact**

Most of the trial plots have been set up on fallows and savannah and are expected to demonstrate that cocoa cultivation is a good way to increase the value of such lands. This should encourage neighbouring farmers who do not own any forest to grow cocoa and those who own forest to spare it and use other land for cocoa cultivation. The activities of old cocoa plots regeneration are also expected to have a positive impact on the limitation of forest use.

Perspectives

The Participatory Breeding Programme will continue with the assessment of the existing trial plots and with the establishment of new plots every year.

Efforts will be made to create new partnerships with local non-governmental organizations (NGOs), which can facilitate the activities through partial funding of the farmers' investments required by the project. In addition, an effort will be made to use the trial plots for improved plantain and fruit tree variety assessment, through partnerships with researchers from IRAD, the World Agroforestry Center (ICRAF) and the International Institute of Tropical Agriculture (IITA).

Of course, the continuation and extension of this Programme will require funding and a constant effort is needed to access different sources of funds.

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Germplasm evaluation and breeding for moniliasis and black pod resistance at CATIE in Costa Rica

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Introduction

The Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) participated in the CFC/ICCO/Bioversity project in the following areas: introduction and exchange of germplasm, evaluation of resistance of germplasm (co-financed by the World Cocoa Foundation, WCF) and participation in the Regional Variety Trial (RVT). These activities were complementary to the large breeding programme carried out by CATIE with support of WCF, the United States Department of Agriculture (USDA) and MARS Inc. Among other activities, the breeding programme has selected six clones for high yield and low moniliasis incidence in 2003. These clones are now being recommended to the cocoa growers and have been distributed to six Central American countries. Currently, another 160 clones are being evaluated in a replicated variety trial. Below, only the activities that received support from the CFC/ICCO/Bioversity project are described.

Germplasm evaluation

To categorize appropriate sources of resistance it is indispensable to have an adequate source of cocoa genetic diversity and reliable methodologies to identify resistant genotypes. CATIE met both requirements (Phillips-Mora et al. 2009):

a. The CATIE International Cocoa Genebank (IC3), one of only two worldwide, currently contains 1107 accessions with different geographic and genetic origins. A continuous strategy for the physical and genetic enrichment of this genebank has been implemented during the last 6 years, with more than 300 wild clones introduced from Reading (UK), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) and several countries in Latin America.
b. CATIE has also developed and implemented effective artificial inoculation methods to test the reaction against moniliasis and black pod. Clones resistant to frosty pod rot (FPR or moniliasis) and/or black pod disease (BPD) were identified in the IC3 by using artificial inoculation techniques developed at CATIE. The latter activity has been identified as a co-financing activity receiving WCF funding.

Moniliasis inoculations are performed using the methodology summarized by Phillips-Mora et al. (2005). So far, 746 clones have been tested against moniliasis and classified as follows: resistant 2%, moderately resistant 8%, moderately susceptible 23% and susceptible 67%.

The most resistant clones are listed below (Phillips-Mora et al. 2009):

ACT 211	IMC (27, 54, 55, 60)
AMAZ 3-2	Laranja
Amelonado 15(6)	México (10, 12A1, 14A1)
ARF (2, 5, 6, 33, 37)	ML 102
BE 8	NA 756
CC 240	Nacional 1 (A3, A4, A6, A13, A14)
CHUA0 120	Nacional 2 (A19, A27, A38)
CL 19/10	P 23
Criollo (8, 14, 43, 66)	PA (44, 67, 169, 303)
EET (75, 129, 401, 407)	Playa Alta 2
EQX 69	PMCT (12, 15, 16, 44, 46, 48, 51, 53, 82)
GC 29	Santa Clara 3
GU (123-N, 125-N, 147-N, 171-N, 254-A)	SC 24
HY 2714184	SGU 84
ICS (10, 75, 95)	UF (273 T1, 613, 712)

The continuous introduction of wild clones into IC3 has permitted the identification of novel sources of resistance within various geographic origins, which are being successively incorporated into the breeding programme. The maintenance of CATIE's genebank and the introduction and evaluation of new germplasm was co-financed by WCF.

The Paper Disc Method (Phillips-Mora and Galindo 1989) is used to test the reaction of the clones to black pod. To date, 819 clones have been evaluated against black pod, obtaining the following distribution: highly resistant 4%, resistant 12%, moderately resistant 15%, moderately susceptible 18%, susceptible 15% and highly susceptible 37%. The clone PMCT 46 registered a high resistance against both pathogens. The following clones were rated as highly resistant to black pod (Phillips-Mora et al. 2009):

APA 5	ML 103
ARF (12, 14, 22, 24, 31, 32)	Nacional 3A41
BE 4	PA (4, 51)
Criollo 34	PMCT (23, 35, 37, 46, 92, 93, 99)
EET (59, 272)	Pound 7
ICS 47	RB 46
México 4A1	SNK 12

Regional Variety Trial (RVT)

In Central America as in other regions, the narrow genetic base of most commercial plantations and their vulnerability to highly destructive pathogens make the development of new varieties with disease tolerance and high yields imperative.

The Regional Variety Trial (RVT) was initiated in La Lola in August 2004. The trial comprises 19 inter-clonal crosses, 6 produced at CATIE and the remaining in Brazil, Ecuador, Peru and Venezuela. The foreign crosses were received at different dates, consequently their establishment in the field was performed accordingly but always after a safe quarantine period. For data analyses, we divided the plants into two planting dates:

- First planting: from 19 August 2004 to 27 January 2005;
- Second planting: from 31 May to 15 November 2005.

First planting

For the first planting the cross Catongo x Pound 12 showed the highest average yield with 645 $kg.ha^{-1}.yr^{-1}$. This cross was used as a local control because of its good production potential, despite the fact that the parents are susceptible to black pod and moniliasis. The second most productive cross was CCN 51 x TAP 12 from Ecuador, which showed an average yield of 525 $kg.ha^{-1}.yr^{-1}$. In both cases, these productivities are considered high, due to the young stage of the trees (5 years old) and the high natural pressure of moniliasis in the area. The cross that had the lowest productivity was EET 183 x (Pound 7 x UF 273 [isla 3 tree 45]) from CATIE with 243 $kg.ha^{-1}.yr^{-1}$.

We identified a group of 14 outstanding trees (2% of the trees studied) due to their high production and low incidence of moniliasis. These trees will be vegetatively propagated to establish a clone trial to verify their behaviour under replicated experimental conditions.

In general, there was a low incidence of moniliasis in the trial, which may be explained by the juvenile state of the plantation and the presence of crosses with resistant genes. The cross with the lowest incidence to moniliasis was (EET 233 x EET 399) x IMC 67 with 13.4%, followed by cross PA 121 x EEM 001 with 21.8%. The crosses with the highest incidence were CP 40 x CP 87 (35.4%) and Pound 12 x Catongo (29.8%), produced respectively in Brazil and CATIE (local control). It is important to note that cross Catongo x Pound 12 had the highest production of dry cocoa but is susceptible to moniliasis and thus not recommended for use in areas with high incidence of the disease. No statistical differences were found for the incidence of black pod between the crosses tested. Incidence levels were very low and ranged between 0 and 2.3%.

Second planting

For the second planting date, the treatment with the highest yield was EET 233 x U 1 (produced in Peru) with 652 kg of dry cocoa per hectare in the first two years of harvest. The second most productive cross was CCN 51 x EET 233 (produced in Ecuador) with 443 kg of dry cocoa/ha. The crosses which produced less were (IMC 67 x U 68) x ICS 95 with 133.98 and PA 169 x ICS 95 with 218 kg dry cocoa/ha, respectively.

In the population (269 trees) 15 superior trees (5% of the population under study) were identified for their high yield and/or resistance to moniliasis; these trees will be propagated vegetatively to corroborate their potential. Of the 15 selected trees, 11 correspond to EET 233 x U 1, confirming that within this progeny there are trees with a high production potential, which deserve to be cloned. Two trees belong to CCN 51 x TSH 1188 (Brazil), one tree to IMC 11 x EEM 008 (Venezuela), and the remaining to (IMC 67 x U 8) x CCN 51 (Peru).

The lowest incidence of moniliasis was observed on trees belonging to EET 233 x U 1. The clone EET 233 has been reported in Ecuador as resistant to moniliasis and this study also showed that it produces an offspring with a very good yielding potential. The combination of the clone EET 233 with Ucayali 1 is thus a very interesting cross for the CATIE´s breeding programme. The second cross in importance is IMC 11 x EEM 008 due to its low incidence of moniliasis (12.3%). The crosses with the highest incidences of moniliasis were CCN 51 x TSH 1188 from Brazil and (IMC 67 x U 68) x ICS 95 from Peru.

Segregating populations

Two segregating populations were established in 2006: EET 183 x (UF 273 x Pound 7 [isla 3 tree 45]) and TSH 1188 x CCN 51. Trunk diameter at 0.30 m was recorded twice a year (December and June). We also evaluated the jorquette height. It will be difficult to continue with the assessment of these populations due to lack of funds.

Conclusions

Major benefits, research needs and perspectives

This project has been very successful in promoting the exchange of valuable germplasm among the participating countries. The Regional Variety Trial gave us the opportunity to introduce into Costa Rica 12 outstanding crosses in terms of their yield and disease resistance potential. We also produced seven relevant crosses concentrating genes of interest. After 2 or 3 years of exhaustive evaluation, we have identified crosses and individual trees with a very promising combination of desirable characters. This happened in spite of the high natural incidence of moniliasis in the experimental area. In particular, the cross EET 233 x U 1 showed a high yield (651 $kg.ha^{-1}.yr^{-1}$) and a very low moniliasis incidence (10%) during its two first two production years. A high number of outstanding trees were selected from this cross. The cross Catongo x Pound 12 registered a high yield potential, however, due to its susceptibility to moniliasis it is only recommended for areas where this disease is not present. The two segregating populations established under the framework of the project remain to be molecularly analysed; however, basic observations and general maintenance of the area have been performed during several years.

Conclusions and recommendations

Artificial methods of inoculation have proven to be effective tools for both determining the reaction of cocoa genotypes to diseases and selecting resistant genotypes. Resistances to moniliasis and black pod segregate independently in cocoa. However, it is feasible to obtain individuals possessing simultaneously high levels of resistance to both diseases.

Introduction of Ucayali 1 from Peru to re-do the cross EET 233 x U 1 is a priority. We also would like to clonally propagate the best trees from the Regional Variety Trial to preserve them, and if funds would be available, to establish them in a clonal trial where their performance could be compared with the best CATIE selections.

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Selection of new varieties on-station and on-farm in Côte d'Ivoire

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Abstract

The CFC/ICCO/Bioversity project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* represents an effort to:

- Validate on-farm improved varieties selected by the Centre National de Recherches Agronomiques (CNRA);
- Explore the results obtained in the project on *"Cocoa Germplasm Utilization and Conservation: a Global Approach"*;
- Promote exchange of varieties with resistance to *Phytophthora* pod rot.

The activities undertaken over the 5-year period (2004-09) included:

- On-station selection of the following trials: two clone trials, two intra-population hybrid trials, three hybrid trials, and the establishment of a regional hybrid trial;
- On-farm survey about the planting materials used by the farmers and the establishment of 36 demonstration plots on farms including 15 improved varieties selected by CNRA and 15 farmers' selections.

The traits evaluated were yield, yield efficiency, percentage of rotten pods and the average weight of one pod. The analyses of the on-station trials showed highly significant differences among the genotypes tested, many of which showed superiority with regard to the control varieties. Genetic parameters were calculated and discussed for the intra-population hybrid trials. This permitted to apply a selection index to increase selection efficiency in these trials. A list of elite hybrids that are candidates for multilocation variety trials has been established. The first results of the Regional Variety Trials shows superiority of the Côte d'Ivoire hybrids for vigour and precocity compared to the introduced varieties.

The farm survey included 1133 farmers over the six main cocoa-producing regions. The results show that most farmers use their own farm or neighbours' farms as sources of new planting materials and that yield and precocity are the main selection criteria. The relevance of establishing demonstration plots on-farm to stimulate the farmers to adopt breeders' selections as well as to identify superior farm selections is presented.

Introduction

In Côte d'Ivoire the breeding programme started in the 1960s with selection of bi-parental crosses between Upper Amazon (UA) and Lower Amazon (LA or Amelonado) or Trinitario (T) parental genotypes. The first series of selected hybrids included 12 hybrid families that were released from 1975 onward (Besse 1977). A second batch of seven hybrids was selected according to the same principle (Paulin et al. 1993; Clément et al. 1999). The criteria for selection of these hybrids were yield, precocity and quality (bean size and fat content).

Afterwards, resistance to *Phytophthora* pod rot (Ppr) and to mirids have become important criteria in Côte d'Ivoire. Since 1990, reciprocal recurrent selection (RRS) based on improvement of an Upper Amazon population (UA) on one side and on Lower Amazon plus Trinitario (LA+T) population on the other side has been adopted, with the following selection criteria: yield, yield efficiency, resistance to Ppr and to mirids, and technological quality. The first cycle of RRS was finalized in 1999 with the selection of the parents for the second cycle of intra-group parents and of seven candidate hybrids for varietal output (Lachenaud et al. 2001).

Between 1998 and 2003 the CFC/ICCO/IPGRI project on *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* has permitted to launch the second cycle of RRS trials for which the field observations are still ongoing. Between 2004 and 2009, the CFC/ICCO/Bioversity project on *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* has permitted to continue the on-station trials and carry out on-farm selection activities with the following objectives:

- Validate, at the farm level, the value of the selected varieties for yield, resistance to diseases and pests, and quality traits;
- Exploit fully the results from the CFC/ICCO/IPGRI project on *"Cocoa Germplasm Utilization and Conservation: a Global Approach"*;
- Promote the exchange of cocoa varieties in the region; and
- Improve the methods used to evaluate resistance to mirids and to Ppr.

The main results of the on-farm and on-station variety trials are presented here.

Materials and methods

On-station selection activities

- **Clone trial**

A local clone trial using rooted cuttings was established in 2000 with 20 clones in a randomized block design with six blocks of eight trees per clone (Table 1). The clones were selected based on yield traits, resistance to Ppr and to mirids.

Table1. Genetic origin of 20 "local" clones (LA = Lower Amazon, UA Upper Amazon)

Clones	Origins	Clones	Origins
IFC 5 (control)	Forastero (LA, Amelonado)	ACT 4-2	Trinitario
IFC 414	Forastero (LA, Amelonado)	WA 40	Trinitario
MO 81	Forastero (UA)	C 151-61 (control)	(SCA 6 x ICS 1) x ICS 1
EQX 94	Forastero (UA)	D 15/2 L26 A9	Hybrid clone
PA 150	Forastero (UA)	D 15/2 L2 A3	Hybrid clone
PA 7	Forastero (UA)	D 15/2 L95 A2	Hybrid clone
P 19	Forastero (UA)	C 2/1 L235 A11	Hybrid clone
T 79/501 (control)	Forastero (UA)	C 2/1 L330 A9	Hybrid clone
UPA 413	Forastero (UA)	C 2/1 L333 A1	Hybrid clone
ICS 39	Trinitario	C 2/1 L120 A2	Hybrid clone

- **Intra-population selection and hybrid family trials**

As part of the RRS programme, several trials were established (Table 2) with the following objectives:

- Two trials with intra-group crosses (E6/1 and E6/4) with 40 parental genotypes for both the UA and LA+T groups, with the objective to accumulate favourable alleles for yield, resistance and quality;
- One hybrid trial with inter-group crosses (E6/5), aiming at recombining genes from different sources;
- One hybrid trial (G15) aiming at evaluating new crosses with resistance to *Phytophthora megakarya*, which is present in East Côte d'Ivoire;
- One hybrid trial with intra-group crosses already present in E6/1 and E6/4 and selfings aiming at concentrating favourable alleles for resistance to Ppr and decreasing the level of heterozygosity in the following selection cycle.

Table 2. Characteristics of trials in the second-cycle recurrent selection, Côte d'Ivoire

Trial and year of planting	Area (ha)	Site	Population	Number of progenies	Trial layout
E6/1, 2000	1.06	Divo	UA2	75 intra-UA crosses and 3 controls crosses	Single tree randomized design; 15 trees/progeny 45 trees/control
E6/4, 2000	1.06	Divo	LA2	70 intra-LA crosses and 3 controls	Single tree randomized design; 16 trees/progeny 46 or 47 trees/control
E6/5, 2002	0.96	Divo	Inter- and intra-population crosses	25 various crosses and 3 controls	Single tree randomized design; 45 trees/progeny 46 or 48 trees/control
G15, 2001	0.86	Abengourou	Inter- and intra-population crosses	22 various crosses and 3 controls	Single tree randomized design; 45 trees/progeny 46 or 48 trees/control
E6/6, 2001	0.77	Divo	Intra-population crosses, selfings	51 various crosses and 2 controls	Single tree randomized design; 15 trees/progeny 40 trees/control
Total	**4.71**	**2**	**5**	**257**	

- **Regional Variety Trial**

Aiming at exchanging varieties with resistance to Ppr and good yield potential in Africa, a regional hybrid trial was established in 2005 with varieties from Cameroon, Ghana and Côte d'Ivoire. The trial of 1.31 ha established in Divo contains 3 blocks and 23 hybrids: 9 from Ghana, 4 from Cameroon and 10 from Côte d'Ivoire.

Variables studied

At least four out of the seven variables indicated below were studied in each trial:

- Average weight of one pod (Pw) in kg;
- Potential cumulated yield (Ypot = Pw x total number of pods) in kg/tree;
- Vigour (Vig) expressed as the trunk radius to the cube (r^3), measured at 20 cm from the soil level for hybrid trees. For clones, vigour was estimated on a 1 to 5 point scale;
- Yield efficiency (Ypot/Vig) in kg/cm^3;
- Percentage of Ppr-infected pods (Ppr%) in relation to the total number of pods produced;
- Bean weight (Bw), estimated by the weight of 100 fermented and dry beans;
- Genetic gain (Gg) calculated between cycle 1 and cycle 2 of the RRS programme.

On-farm selection trials

Two main activities were carried out:

- A farm survey aiming at a better understanding of the planting materials used by farmers and of the knowledge of farmers about their planting materials;
- Establishment of on-farm trials comparing improved varieties with farmers' selections.

- **Farm survey**

The farm survey included 1133 farms located in 262 villages in the main cocoa-producing regions in the country (Table 3; Figure 1). The survey included questions on the origin of the planting materials, the type of planting materials used, the selection criteria applied by the farmers, and on the farmers' knowledge about outstanding trees (for yield and resistance to Ppr and mirids).

Table 3. Number of villages and farmers visited per region where the farm survey was carried out

Regions	Number of villages	Number of farmers
Bas Sassandra	60	188
Haut Sassandra	34	198
Moyen Cavally	30	168
Moyen Comoé	43	219
Sud Bandama	63	161
Sud Comoé	32	199
Total	**262**	**1133**

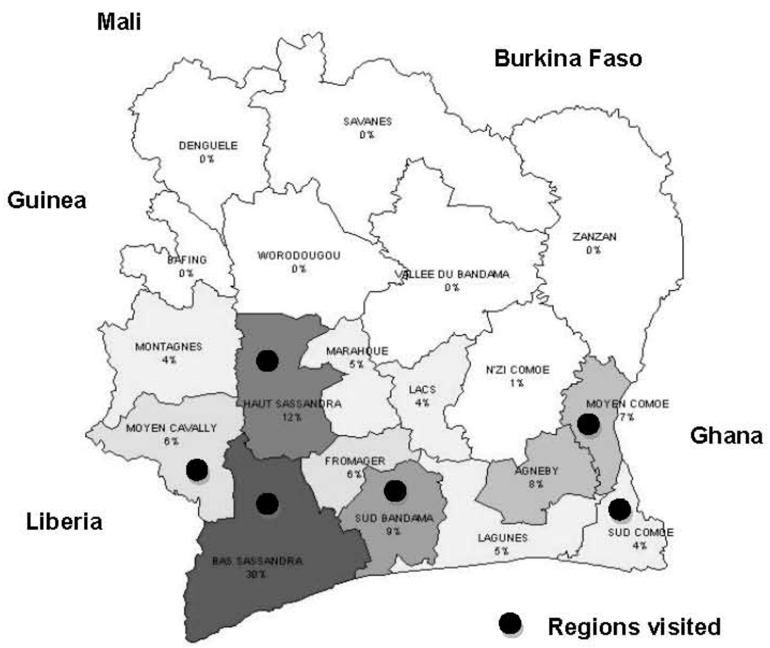

Figure 1. Localization of the six cocoa-producing regions visited during the farm survey (black dots). For each cocoa-producing region, the production is indicated as percentage of national production (source: Ministère de l'Agriculture (Anonyme 2004)). The darkest shading corresponds to the highest production regions while regions in appearing white correspond to marginal regions where the production is very low or even nil.

- **On-farm trials**

A network of 36 plots of 0.29 ha each was created between 2005 and 2009 in five cocoa production areas: Soubré, Agboville, Oumé, Abengourou and Divo. The planting material included pods chosen by the farmers from their best five trees (according to their own criteria) as well as pods from 15 improved hybrids received from the CNRA. Nurseries were established near to water wells in each of the villages.

In each plot 30 varieties are compared including the 15 CNRA hybrids and 15 farmers' selections, proposed by three farmers situated on the same axis (five varieties of each farmer). The varieties were planted in rows of 10 trees for each variety, making a total of 300 trees per plot; a border row is included at all sides of the plot.

After establishment of the plots, some had to be abandoned due to high mortality rates and others due to farmers' neglect. Table 4 shows the number of plots created and the number of plots that are still active in 2010.

The variables observed in the on-farm trials are: mortality rates, number of healthy, rotten or rodent-attacked pods produced per variety, and the preference of the farmer for the best variety.

Table 4. Number of plots established and still active in 2010 in on-farm trials in Côte d'Ivoire

Region	No. of plots of 0.29 ha	Year of planting	No. of plots available in 2010
Soubré	9	2005, 2006, 2009	6
Agboville	9	2006, 2007	6
Oumé	9	2006, 2007	5
Abengourou	7	2007, 2008	6
Divo	2	2008	2
Total	**36**		**24**

Results and discussion

On-station trials

- **Local clone trial**

Five traits were studied: Ypot, Vig, Ppr%, Pw and Bw. For the Ypot and Ppr% the analysis is based on six harvesting years (2002 to 2008). Main results are presented in Table 5. A significant clone effect was observed for all traits studied.

Table 5. Performances of local clones with regard to yield, vigour, percentage of rotten pods and bean weight

Traits	Average			
	Best clone	Worst clone	Amplitude of the 5 best clones	Best control
Ypot (kg.ha^{-1}.year^{-1})	3440	382	3440-2314	3440 (T79/501)
Vig (by scoring)	4	1.9	4-3.2	3.3 (T79/501)
Ppr%	6	28	6-8	5 (C151-61)
Pw (kg)	0.63	0.31	0.63-0.47	0.48 (T79/501)
Bw (g)	182.9	95.3	182.9–123.1	115.3 (IFC5)

Besides the control varieties (T79/501 and C151-61), some clones such as D15/2 L95 A2 and C2/1 L120 A2 selected in hybrid families are among the best clones for the majority of traits measured. The results suggest that the following clones could be used in further breeding:

- T 79/501, C 151-61, D 15/2 L 26 A9, D 15/2 L95 A2 and C 2/1 L120 A2 for their yield potential,
- T 79/501, C 151-61, D 15/2 L26 A9 and C 2/1 L235 A11 for field resistance to Ppr,
- ICS 39, ACT 4-2, IFC 414, WA 40, D 15/2 L2 A3 and C 2/1 L120 A2 for 100-bean weight.

- **Second cycle of RRS**

Between 2002 and 2008, the following traits were evaluated: Ypot, Ypot/Vig, Ppr%, and average Pw. Analyses were done by family and also according to the factorial design of the trial (two factors: male and female).

Analyses of hybrid families in the populations UA2 and (LA+T)2

For each of the traits, the effect of families was highly significant, with homogeneous groups ranging from 4 to 14 depending on the trait. Main results are presented in Tables 6 and 7.

Table 6. Averages of the progenies in the UA2 population

Traits	Average			
	Best progeny	Worst progeny	Amplitude of the 5 best progenies	Best control
Ypot ($kg.ha^{-1}.year^{-1}$)	2494	989.5	2494-2230	1899.2
Ypot/Vig (kg/cm^3)	0.46	0.14	0.46-0.39	0.27
Ppr% (%)	5	17	5-6	8
Pw (kg)	0.60	0.34	0.60-0.54	0.56

Table 7. Averages of the progenies in the (LA+T)2 population

Traits	Average			
	Best progeny	Worst progeny	Amplitude of the 5 best progenies	Best control
Ypot ($kg.ha^{-1}.year^{-1}$)	2208	142	2208-1444	1417.1
Ypot/Vig (kg/cm^3)	0.33	0.04	0.33-0.27	0.22
Ppr% (%)	3	34	3-7	14
Pw (kg)	0.61	0.27	0.61-0.56	0.52

For the best five families, the yield potential and yield efficiency vary in the UA2 population between 2494 to 2230 $kg.ha^{-1}.year^{-1}$ and from 0.46 to 0.39, respectively (Table 6), while the same traits varied in the (LA+T)2 population from 2208 to 1444 and 0.33 to 0.27, respectively. These differences show that the UA2 population contains the better families. For the majority of the traits, the best five families in each population are better than the best control variety, which is one of the commercial hybrids with highest yielding capacity currently distributed to farmers in Côte d'Ivoire. These results suggest that important progress is possible in the second cycle of the RRS programme.

Analyses of the female and male effects

Both the male and female effects were highly significant for all traits observed except for Ppr% in the (LA+T)2 population (Table 8). The female x male interaction effect was never significant, except for the yield potential in the (LA+T)2 population.

Table 8. Analysis of parental effects in the UA2 and (LA+T)2 populations for four selection traits

Traits	UA2			(LA + T)2		
	Significance			Significance		
	♀	♂	♀ x ♂	♀	♂	♀ x ♂
Ypot (kg.ha^{-1}.year^{-1})	***	***	ns	***	***	***
Ypot/Vig (kg/cm^{3})	***	***	ns	***	***	ns
Ppr% (%)	***	***	ns	***	ns	ns
Pw (kg)	***	***	ns	***	***	ns

*** = highly significant
ns = not significant

Correlations between traits and individual tree narrow-sense heritabilities

Table 9 shows the phenotypic correlations (upper part) and the additive genetic effects (lower part) as well as the narrow-sense heritabilities (diagonal) for both UA2 and (LA+T)2 populations.

The yield traits are phenotypically and genotypically correlated in both populations. The genetic correlations (between means per family) are higher for the (LA+T)2 population than for the UA2 population.

A non-significant negative correlation (r=-0.13) is observed between Ypot and Ppr% for the UA2 population, whereas this correlation is highly significant for the (LA+T)2 population (r=0.83). This suggests that the best parents in the (LA+T)2 population for yield transmit also the highest susceptibility to Ppr. In both populations a significant correlation between the Pw and Ppr% is observed, indicating that the size of the pod is a factor that favours Ppr infection levels in the field.

With regard to the narrow-sense heritabilities, the highest values were observed for Pw in the UA2 population (0.52) and in the (LA+T)2 population (0.24), and for the yield efficiency (Ypot/Vig) with heritabilities of 0.25 for the UA2 population and 0.42 for the (LA+T)2 population.

Table 9. Phenotypic (above diagonal) and genetic (below diagonal) correlations between traits, and narrow-sense heritabilities (on the diagonal) for yield (Ypot), yield efficiency (Ypot/Vig), *Phytophthora* pod rot (Ppr%) and pod weight (Pw) in UA2 and (LA+T)2 populations

Traits	UA2 population				(LA+T)2 population			
	Ypot	Ypot/Vig	Ppr%	Pw	Ypot	Ypot/Vig	Ppr%	Pw
Ypot (kg.ha^{-1}.year^{-1})	**0.16**	0.80	-0.39	-0.02	**0.18**	0.93	-0.28	0.15
Ypot/Vig (kg/cm^{3})	0.49	**0.25**	0.38	0.17	0.95	**0.42**	-0.25	-0.02
Ppr% (%)	-0.13	-0.34	**0.17**	0.60	0.83	0.67	**0.05**	0.05
Pw (kg)	-0.24	-0.17	0.71	**0.52**	0.65	0.61	0.73	**0.24**

These values can be considered as relatively high, which can be partly ascribed to adjustments applied to the original data based on the method of Papadakis (1937), which helps to decrease the effect of the environment. The two traits with highest narrow-sense heritabilities (Ypot/Vig and Pw) show therefore predominance of additive genotypic effects, as was shown also by the larger part of the genetic variance in relation to the dominance variance for both of these traits (data not shown).

With regard to the heritability of 0.17 for Ppr% in the UA2 population, the heritability for the (LA+T)2 population is nearly zero (0.05). This demonstrates that the dominance effects are significant and the transmission of this trait in the (LA+T)2 population is limited. This result is coherent with the small genetic gain observed for Ppr% between the first and the second cycle (LA+T) populations (-7.2%).

Genetic gains and selection index

The genetic gains for Ypot, Ypot/Vig, Ppr% and Pw were calculated with a selection intensity of 1% (Table 10). The selection index that was applied with the following weights: 1 for Ypot, 3 for Ypot/Vig, -5 for Ppr% and 2 for Pw, aimed at strong progress for resistance to Ppr and for yield efficiency. Table 10 shows that the expected genetic gains were 41.3% for Ypot, 29.7 % for Ypot/Vig, -27.7% for Ppr% and -13.3% for Pw for the UA2 population, while the same genetic gains for the (LA+T)2 population were 165.6%, 136.3%, 48.1% and 29.6%, respectively.

The negative genetic gain for Ppr% in the UA2 population indicates an increase in resistance by applying the selection index, whereas the positive genetic gain for the same trait in the (LA+T)2 population indicates an increase in susceptibility by applying the selection index. The increase in resistance in the UA2 population is causing a slight decrease in Pw (-13%). Also, the increase in Pw in the (LA+T)2 population is accompanied by a strong increase in yield potential and yield efficiency.

In conclusion, the genetic parameters calculated in the above manner permit constructing a selection index with the objective to select for:

- Good yield potential with high resistance to Ppr and a moderate vigour in the UA2 population,
- High yield potential with moderate vigour and good quality (pod size) in the (LA+T)2 population.

Table 10. Genetic gains in UA2 and (LA+T)2 populations for yield (Ypot), yield efficiency (Ypot/Vig), *Phytophthora* pod rot incidence (Ppr%) and pod weight (Pw) and selection of the best parents selected by applying a selection index

Traits	**Weight**	**Genetic gains (%) for 1% selection intensity**	
		Population UA2	**Population (LA+T)2**
Yield (Ypot)	1	41.3	165.6
Yield efficiency (Ypot/Vig)	3	29.7	136.3
Phytophthora pod rot (Ppr%)	-5	**-27.7**	**48.1**
Pod weight (Pw)	2	-13.3	29.6
Best parents (selection index)	Male	BL9-2, E4/1-6, C2/1-1, E4/3-1, C2/1-3	DCG1-1, E4/2-21, E4/2-14, E4/2-17, E4/2-23
	Female	E4/1-15, E4/1-14, E4/3-2, E4/1-16, E4/1-12	E4/2-7, E4/2-31, E4/2-16, E2/3-2, E4/2-29

Comparison of selection progress between the first- and second-cycle populations in the RRS programme

The four populations considered in this study are UA1, UA2, (LA+T)1 and (LA+T)2. The number of families studied, number of trees per family and number of harvests are indicated in Table 11.

Table 11. Number of progenies analysed per population in each selection cycle

Population	Plot (year of planting)	No. of families per population	No. of trees per progeny	No. of harvests
UA1	E4/1 (1992)	78	20	4
(LA+T)1	E4/2 (1991)	116	24	4
UA2	E6/1 (2000)	75	15	5
(LA+T)2	E6/4 (2000)	70	16	5

The analyses of variance show significant differences between populations for the four main selection traits (Table 12). The genetic gains are also indicated in the same table.

Table 12. Comparison of UA1, UA2, (LA+T)1 and (LA+T)2 populations for four selection traits and genetic gains between the first and the second cycle of intra-population crosses

	Selection traits[1]							
Population	**Ypot**		**Ypot/Vig**		**Ppr%**		**Pw**	
UA1	15.20	a	0.46	a	16	c	0.41	a
UA2	16.00	a	0.47	a	7	a	0.44	a
(LA+T)1	4.90	b	0.25	b	14	bc	0.36	b
(LA+T)2	5.10	b	0.22	b	13	b	0.42	a
First to second cycle	**GG (genetic gains) expressed in %**							
UA1 to UA2	5.3		2.2		-56.2*		7.3	
(LA+T)1 to (LA+T)2	4.2		-12		-7.1		16.7*	

[1] Values followed by the same letter are statistically equal according to the Newman and Keuls test at 5% probability.
* = significant at 5% probability

For the UA population, a significant decrease in Ppr incidence is observed between the two selection cycles. This is not unexpected, as resistance to Ppr has been an important selection criterion. For the (LA+T) populations, an increase of 16.7% in Pw has been observed between the two cycles. This suggests that the Trinitarios have been useful to increase pod size and also bean size, which are correlated traits in this population.

Hybrid trial and inter-group test

Twenty-five crosses between different genetic groups (UA, LA and GU) were planted in a trial in 2001 (E6/5). The parents were selected for their resistance to Ppr, as evaluated by the leaf disc test. Each family contained 45 trees, except the 3 control families which contained between 50 and 55 trees. For all four selection traits, the family effect was highly significant. The superiority of inter-group crosses over intra-group crosses was evident in this trial (see Table 13). The best families for yield, yield efficiency and resistance to Ppr have one GU parent, indicating the benefit to use more French Guiana parents in cocoa breeding in Côte d'Ivoire.

Table 13. Analysis of inter- and intra-population crosses

	Traits			
	Ypot ($kg.ha^{-1}.year^{-1}$)	**Ypot/Vig** (kg/cm^3)	**Ppr%**	**Pw** (kg)
Origin of the 5 best progenies	UA x UA	LA x GU	UA x GU	UA x T
	LA x GU	T x GU	LA x GU	UA x (LA+T)
	UA x GU	LA x GU	T x GU	UA x HA
	UA x (LA+T)	UA xUA	UA x GU	UA x (LA+T)
	UA x GU	T x GU	UA x GU	UA x GU
Mean of the best progeny	2071	0.39	4	0.6
Amplitude of the 5 best progenies	2071-1808	0.39-0.32	4-6	0.60-0.48
Mean of the best control	1546	0.23	8	0.60

Selection of promising trees for Ppr resistance and pod weight in some selfed progenies

Fifty-three families including two selfed progenies of UA1 parents, nine selfed progenies from (LA+T)1 parents and forty-two families from UA2 and (LA+T)2 populations (already planted in E6/1 and E6/4) that had shown good resistance to Ppr in the leaf disc test, were evaluated in one trial planted in 2000 (Divo E6/6). The analyses show inferiority of the majority of the selfings for potential yield and for yield efficiency. However, with regard to Ppr incidence, very interesting trees can be selected (Table 14). In one other selfed progeny, trees with high pod weight could be selected.

Table 14. Selection of promising trees in some progenies obtained by selfing

Selfing	No. of trees	Mother plant selected for	Amplitude		Observations
			Ppr%	Pw (kg)	
501	16	Resistance to Ppr	0-15%	0.12-0.37	12 trees with 0<Ppr<3%
601	12	Resistance to Ppr	0-42%	0.15-0.45	8 trees with Ppr=0%
605	12	Resistance to Ppr	0-20%	0.12-0.59	9 trees with Ppr=0% 3 trees good for Ppr and Pw
606	14	Pod weight	0-44%	0.41-1.00	13 trees with Pw>0.53 Kg
607	12	Yield	0-52%	0.12-0.59	9 trees with 0<Ppr<4%
608	12	Resistance to Ppr	0-3%	0.20-0.54	All trees good for Ppr

Regional Variety Trial

Twenty-three hybrid families, including nine from Ghana, four from Cameroon and ten from Côte d'Ivoire were planted in trial E8/2 in 2005. After 2 years of planting, the Côte d'Ivoire hybrids showed to be more vigorous. Four years after planting observations were analysed regarding Ypot, Ypot/Vig, Ppr%, Bw and Pw (Table 15). The analyses of variance showed significant differences for all traits observed. The Côte d'Ivoire hybrids showed superiority for Ypot, Ypot/Vig, Bw and Pw, but the families from Ghana and Cameroon were superior for Ppr%.

Table 15. Preliminary results obtained with hybrid families originating from Côte d'Ivoire (CI), Ghana (GH) and Cameroon (CA) for Ypot, Vig, Ppr%, 100-bean weight (Bw) and Pw

	Traits				
	Ypot (kg/ha)	**Vig (cm)**	**Ppr (%)**	**Bw (g)**	**Pw (kg)**
Origin of the best 5 progenies	CI (1729.7)	CI (36.7)	CA (4)	CI (133.6)	CA (0.64)
	CI (1636.4)	CI (35.6)	GH (5)	CI (133.4)	GH (0.61)
	GH (1166.4)	CI (35.4)	CA (5)	CI (132.1)	CI (0.54)
	CI (1084.7)	CA (34.8)	GH (5)	CA (129.2)	CI (0.52)
	CI (1007.7)	CI (33.6)	GH (6)	CI (127.2)	GH (0.50)
Origin of the worst 5 progenies	CI (222.8)	GH (25.5)	CI (14)	CI (91.1)	CA (0.35)
	CA (268.3)	GH (26.8)	GH (12)	CI (102)	CA (0.36)
	GH (419.9)	CA (27.1)	CI (11)	GH (103.5)	GH (0.37)
	CI (513.2)	GH (27.8)	CI (11)	CI (104.6)	CI (0.37)
	CA (536.5)	CI 28.1)	CI (10)	CI (108.5)	CI (0.37)
Mean of trial	**793.1**	**31.1**	**8**	**144.6**	**0.44**

On-farm selection activities

- **Planting material used by the farmers**

The analyses of the farm survey indicate that the farmers obtained planting materials from seven different sources (Table 16). The majority of the farmers used seeds obtained from farmers' fields (parents, neighbours or friends). Only 16.1% of the farmers used improved varieties obtained from research stations or extension services.

Table 16. Origin of seed used by farmers in Côte d'Ivoire

Origin of seed	% of farmers
1. Parents	46.6
2. Neighbours	42.0
3. Friends	20.1
4. Own previous farms	10.4
5. Research Station	9.0
6. Extension service	7.8
7. Local market	2.5

- **Knowledge of farmers about their planting materials**

The farmers identified three types of varieties:

1. 'Cacao Français' (French Cocoa). This is the traditional 'Amelonado' variety introduced first into Côte d'Ivoire.
2. Improved hybrid varieties. These were identified by the farmers as 'IFCC', 'SATMACI', '18 months cocoa' and, more recently, 'Mercedes cocoa'. These varieties were characterized as being early-yielding, high-yielding, having relatively big beans and a high number of beans per pod.
3. 'Tout venant', or planting material of unknown origin.

- **Proportion of farmers using known varieties**

The proportion of farmers who use a specific type of planting material has been estimated for each of the five main cocoa-growing regions in Côte d'Ivoire (Figure 2). It confirms that in all regions the improved hybrid varieties are used in low proportions. The predominantly used variety is 'Tout venant', especially so in the Cavally and Sud Comoé regions. The cocoa plantations in Côte d'Ivoire are therefore very heterogeneous, with mixtures of 'Tout venant', 'Amelonado' (rare) and improved hybrids (also rare).

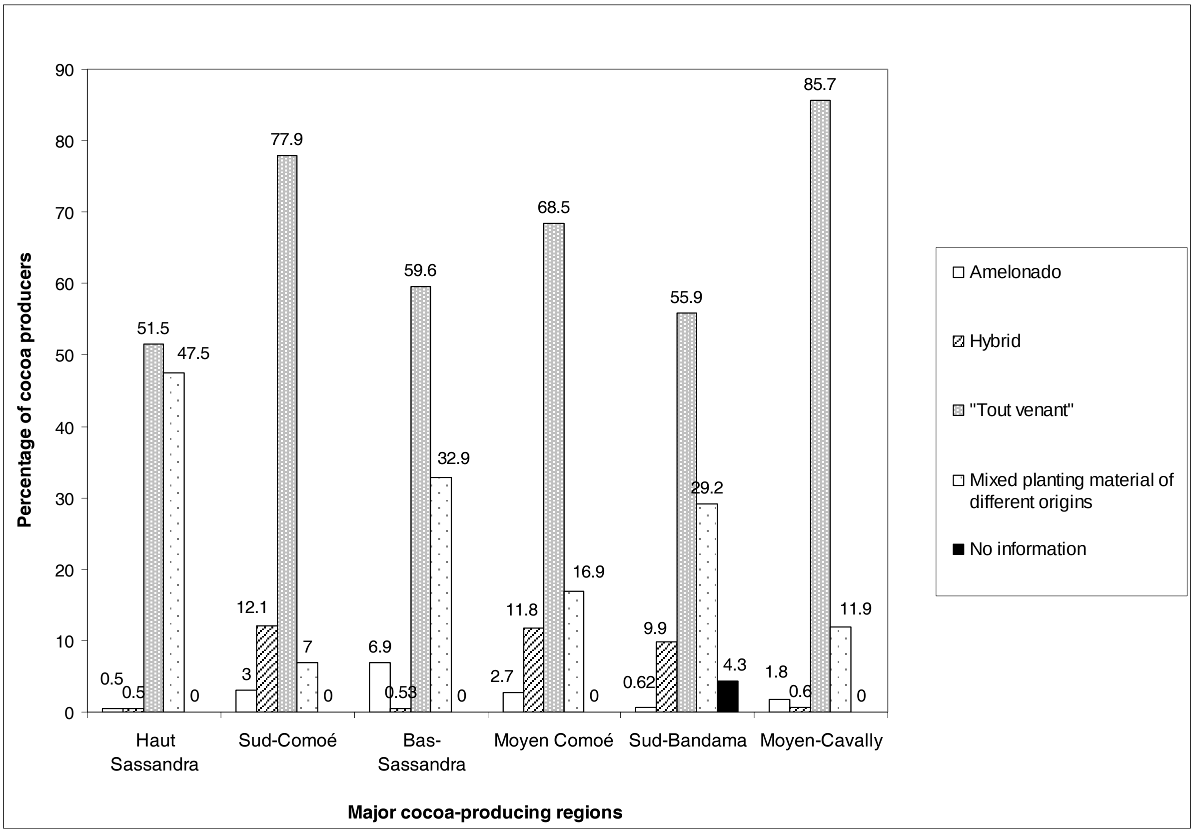

Figure 2. Proportions of farmers using different types of varieties in six cocoa-growing regions.

- **Criteria applied by the farmers in their choice of planting materials**

Table 17 shows several criteria that the farmers judge as important in selecting their planting materials. The three most important criteria are high yield, precocity and bean size. The colour of the pods and size of the trees are criteria of lesser importance to the farmers. Resistance of the trees to drought, swollen shoot, mirids and black pod are becoming increasingly more important criteria.

Table 17. Criteria used by farmers in choosing new planting material

Criteria for choosing planting material	% of farmers
1. High-yielding	94.4
2. Early-bearing	85.6
3. Trees having pods with large beans	84.4
4. Vigorous trees	76.0
5. Trees having big pods	73.7
6. Drought-resistant trees	69.5
7. Trees with some tolerance to CSSV[1]	66.3
8. Trees showing low mirid damage	58.3
9. Trees showing low black pod level	57.6
10. Short trees	48.0
11. Trees having immature green pods	39.5
12. Trees having immature red pods	34.8
13. Tall trees	32.8

[1] CSSV = Cocoa swollen shoot virus

Demonstration plots established on-farm

Thirty-six demonstration plots were planted between 2005 and 2009 in five cocoa-producing regions (Soubré, Agboville, Oumé, Abengourou and Divo). The plots were established with the active participation of the farmers. Fifteen hybrid families from CNRA are compared to fifteen local farms selections. Each farm selection represents an open-pollinated progeny from a tree selected by the farmers. The farmers used the following selection criteria: yield, low incidence of Ppr and of mirids attacks.

Five years after the establishment of the first trial plots, the number of active plots has decreased from 36 to 24. The evaluation of the plots continues, with three main criteria: survival rates, potential yield and preference of the farmers.

• Survival rate

This trait has been measured in plots at least 3 years old in the Oumé, Abengourou and Soubré regions (Table 18). For the varieties of the CNRA the survival rate was 89% (Soubré) and 78% (Oumé), whereas the farmers′ selections survival rates for these regions were 77.2% and 66%, respectively. In general, the CNRA selections showed higher survival rates, indicating better adaptation to the ecological zones tested.

Table 18. Survival rate in demonstration plots 3 or 4 years after establishment

Region	No. of plots involved	Origin of plant material	Survival rate (%)
Oumé	2	CNRA	78.0
		Farmers' farms	66.0
Abengourou	3	CNRA	78.7
		Farmers' farms	53.5
Soubré	4	CNRA	89.0
		Farmers' farms	77.2

- **Number of pods**

Counting of pods on each variety has been carried out during one year, with two-three harvests per plot. The analyses of variance show highly significant differences between varieties, with the CNRA varieties being most precocious (Table 19). The best families for this trait were:
- F3, F15, F11, F1 and F14 for the Oumé region;
- F6, F10, F7, F4 and F2 for the Abengourou region; and
- F9, F10, F4, F7 and F8 for the Soubré region.

Families F4 and F10 are among the best varieties in the Abengourou and Soubré regions.

Table 19. Comparison between CNRA varieties and farmers' varieties

Region	Origin of plant material	No. of pods collected[1]		STD	Best 5 families per region
Oumé	CNRA	22.0	a	18.5	F3, F15, F11, F1, F14
	Farmers' farms	12.0	b	11.5	
Abengourou	CNRA	10.2	a	18.9	F6, F10, F7, F4 , F2
	Farmers' farms	8.4	b	23.1	
Soubré	CNRA	17.1	a	32.6	F9, F10, F4, F7, F8
	Farmers' farms	3.3	b	10.3	

[1] Values followed by the same letter are statistically equal according to the Newman and Keuls test at 5% probability.

- **Preference of the farmers for the varieties**

Three groupes of farmers varying between 15 and 22 visited one plot in Abengourou and two plots in Soubré. The visits were carried out in the same period of the year. The results are presented in Tables 20 and 21. The main selection criterion of the farmers was high yield.

Table 20. Eight best varieties preferred by 22 farmers in a demonstration plot established in Abengourou

No. of line in the plot	Variety planted on the line	No. of farmers	Reason for choosing the variety
L11	F2 (CNRA)	3	High-yielding and pod size
L12	F6 (CNRA)	3	Pod size, high-yielding
L16	F9 (CNRA)	1	Low mortality level
L21	Farmer's variety	3	High-yielding, low mortality level
L22	F7 (CNRA)	2	Short trees, yielding
L23	F4 (CNRA)	3	Vigorous trees, high-yielding
L26	F10 (CNRA)	4	High-yielding
L29	Farmer's variety	3	High-yielding
CNRA varieties	16		
Farmers' varieties	6		
Total no. of farmers		22	

Table 21. Eight best varieties preferred by farmers in two plots established in Soubré

a. Plot 1 (Diarrassouba)

No. of line preferred	Variety planted on the line	No. of farmers	Reason for choosing the variety
L3	Farmer's variety	3	Low canopy
L5	Farmer's variety	2	Short trees
L6	F5 (CNRA)	2	High-yielding
L9	F4 (CNRA)	4	High-flowering and yielding
L12	F12 (CNRA)	3	High-flowering and yielding
L14	Farmer's variety	1	Vigour
L20	F14 (CNRA)	1	Vigour
L25	F6 (CNRA)	1	Vigour
CNRA varieties		11	
Farmers' varieties		6	
Total no. of farmers		17	

b. Plot 2 (Jacques)

No. of line preferred	Variety planted on the line	No. of farmers	Reason for choosing the variety
L2	Farmer's variety	2	Density of foliage, low canopy
L3	F12 (CNRA)	5	Vigour, high-yielding, pod size
L4	Farmer's variety	1	Vigour
L5	F4 (CNRA)	3	High-yielding, low mortality level
L9	F9 (CNRA)	1	Vigour, high-yielding
L10	F13 (CNRA)	1	Vigour
L11	Farmer's variety	1	Vigour
L19	F15 (CNRA)	1	Low mortality level
CNRA varieties		11	
Farmers' varieties		4	
Total no. of farmers		15	

The results show that the number of preferred varieties of CNRA was higher than the number of farmers' selections. The overall results show that 70% of the farmers showed preference for the CNRA varieties, whereas 30% showed preference for the farmers' selections. It should be noted that some of the farmers' selections had a good performance, indicating that the parental trees of these varieties should be preserved for further breeding purposes.

Conclusion and perspectives

Conclusions

In the local clone trial, many clones (C 2/1 L120 A2, D 15/2 L26 A9, ACT 4-2, IFC 414, C 2/1 L235 A11) showed superiority over certain control clones. These clones represent candidate clones for varietal output as well as for further use in breeding.

Concerning the hybrid trials, the good performance of hybrid families with a wild French Guiana parent suggest that this group could be considered as a third group for the recurrent selection programme. It has been shown also that superior trees can be selected in selfed UA1 progenies, at least for resistance to Ppr. The progress observed between the first- and second-cycle populations of the RRS programme justifies the value of recurrent selection for several of the selection traits. This applied especially to the size of the pods in the (LA+T) population and to Ppr resistance in the UA populations.

The first results from the regional variety trial show best performance for yield, vigour and pod weight of the hybrids from Côte d'Ivoire, but for resistance to Ppr the varieties from Ghana and Cameroon were superior.

Regarding the on-farm selection activities, the survey including 1133 farmers constitutes a valuable database for guiding research and development activities. More knowledge has been obtained on the type of varieties grown in Côte d'Ivoire, on the origin of the planting materials, and on the selection criteria of the farmers for choosing new planting materials.

The establishment of observation plots on-farm has already had a positive effect on the attitude of the farmers towards improved varieties. The organization of field days has permitted increased exchange between farmers and researchers on one side, and between farmers themselves on the other side.

Perspectives

- **Activities to be continued on-station**

The narrow-sense heritabilities and genetic gains obtained for the yield traits (Ypot and Ypot/Vig) and for pod weight (Pw) have shown to be high for the UA and (LA+T) populations. This suggests that further progress is possible through continued inter-group crosses, while at the same time starting to carry out reciprocal recurrent selection.

Trees obtained through selfing of several UA1 parents have shown high field resistance to Ppr. These trees will be cloned to confirm their value for this trait.

A factorial 5 x 5 crossing design including five of the best parents of UA2 and (LA+T)2 populations will be established, including some intra-group crosses as controls. In the same trial, crosses between some of the best parents and three French Guiana clones will be included.

The genetic diversity of the collection will be increased by further introduction of exceptional trees selected in the UA2 and (LA+T)2 populations.

Fifteen intra-group crosses that are part of the second cycle of the RRS programme have been identified to have superior behaviour for yield traits as well as for resistance to Ppr and average pod weight. These crosses will be planted in a multilocation trial aiming at varietal output.

- **Activities to be continued on-farm**

The evaluation of the on-farm trial plots has been irregular and insufficient. It is strongly recommended that these observations continue for a valid exploitation of the results.

The mother trees of the best farmers' selections should be observed and cloned if their behaviour is confirmed.

The organization of field days should continue in the various regions, in order to increase the interest of the farmers in adopting improved varieties for new plantings, according to sound agronomic practices.

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On-farm and on-station selection of new cocoa varieties in Ecuador

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Abstract

The CFC/ICCO/Bioversity International project *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* (2004-2010), chapter INIAP-Ecuador, provided an opportunity to continue evaluating most of the hybrid and clone trials started during the CFC/ICCO/IPGRI project *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* (1998-2003), and to start participative cocoa research with the inclusion of producers pursuing a more efficient selection of superior cocoa cultivars. Under the umbrella of the current project, an agro-socio-economic study was completed, providing some useful insights on the multifactor environment which influences cocoa production in three distinct zones of Ecuador. Some important germplasm collecting activities also took place, enriching the phenotypic and genotypic variability of the cocoa genebank held by the Instituto Nacional de Investigaciones Agropecuarias (INIAP) at the Estación Experimental Tropical Pichilingue (EET Pichilingue). The project's period was also fruitful in providing information on the influence of some agronomic factors (compatibility, pruning levels, planting density, drought resistance, differences in flowering and other phenological markers) and on sensorial differences among distinct genotypes. Best hybrid trees were selected and multiplied as clones to compare their performance in trials which are currently being conducted both at the EET Pichilingue and in farmers' fields. These experiments will hopefully be the source of new commercial fine or flavour cocoa cultivars in the near future. The most important output of both above-mentioned projects is their contribution, together with other projects, towards building up an important genetic base to gradually develop new cocoa varieties well into the next quarter of the century. Training and opportunities to develop research, agronomic and managerial experience for a number of people locally involved in the project represent an additional dimension in which the project made valuable contributions. We may well divide the cocoa breeding process in Ecuador into two parts, i.e. before and after the implementation period of both projects (1998-2010).

Introduction

Cocoa production constraints

To take advantage of its position in the world as the main fine or flavour cocoa producer and exporter, Ecuador needs to overcome obstacles hindering a faster growth of cocoa production. Challenges are various, with productivity and quality development being among the most important. Low cocoa productivity is due to several causes: poor performance of traditional cocoa fields, low-yielding planting material and high vulnerability to diseases (witches' broom and moniliasis). Productivity becomes even more depressed in marginal cocoa-growing areas due to adverse environmental conditions, particularly drought and soil fertility constraints.

Around 80% of the land planted to cocoa is in the hands of smallholders. They think twice before deciding to invest to increase the productivity of traditional cocoa fields. Well-performing plants are at the heart of any production system. If their genetic base is poor regarding high yield and disease resistance, there is a high risk that investments will not bring expected returns. This scenario weakens the will of the producer to keep on investing in cocoa. The supply of genetically improved cocoa cultivars, equipped with a high-yielding potential and less vulnerability to diseases, is therefore key to helping solve the problem of the low average cocoa yield (below 0.3 tons/ha) and the slow growth of fine or flavour cocoa production and exports.

Evolution of cocoa breeding objectives

A host of factors associated to the problem of the low average cocoa yield in Ecuador justifies the urgency to develop new productive fine or flavour cocoa varieties. As stated earlier, witches' broom and moniliasis incidence, combined with a low yielding potential of the planting material present in the traditional cocoa fields, are by far the main factors limiting productivity and production of fine or flavour cocoa. Both diseases represent a depressing factor since their outbreak in the early 1920s, quickly reaching an epidemic dimension. This was the major incentive to launch formal research activities in cocoa, as part of a Cooperation Agreement in 1943 between the Governments of USA and Ecuador.

During the period 1920-1935, diseases cut the cocoa production down to 25% of its original level (from 40 000 tons to around 10 000 tons), creating an unprecedented national economic crisis since it represented at that time more than 60% of the export income. In response to this serious situation, some producers engaged in isolated and individual efforts during the 1930s to select in their cocoa fields those trees showing limited disease incidence. These were further reproduced by seeds to replace the plants destroyed by the diseases. Genetic segregation diluted any benefit that could have been expected from these initiatives to face this sanitary problem. Clonal technology to fix favourable traits of the selected trees was unknown at that time.

These first actions opened the road to visualize and formulate the objectives of cocoa genetic improvement after the institutionalization of a cocoa research programme based at the Hacienda Pichilingue, which became later the Estación Experimental Tropical Pichilingue (EET Pichilingue) of the Instituto Nacional de Investigaciones Agropecuarias (INIAP). These objectives were adjusted over time and are summarized as follows:

1. To set up a germplasm bank with sufficient genetic variability to increase the opportunities to select breeding material or genotypes worth further development as clonal cultivars;
2. To increase the allelic frequency of genes associated to high yield and disease resistance traits in breeding populations;
3. To create breeding populations to select hybrids and clones which combine high yield potential and disease resistance;
4. To incorporate the criteria of integral quality for additional screening of these selections;
5. To develop and release to farmers superior cocoa varieties equipped with a combination of attributes, mainly disease resistance, high yield and inherent quality, placing emphasis on fine or flavour cocoas.

The activities carried out in Ecuador by INIAP in the framework of both the CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects contributed through different ways to the progress made in reaching the above objectives. The genetic base of the local genebank was widened and enriched through the introduction of foreign and local cocoa germplasm. Hybrid populations were obtained through several crossing schemes, with a possible increase of the frequency of alleles associated to disease resistance, high yield and better quality standards. A close examination has revealed opportunities to select superior hybrid trees, which have been multiplied as clones to compare their performance in formal trials.

On the other hand, studies conducted on the physical and sensorial properties of many of the trees making up the hybrid populations generated useful data to visualize a wide range of opportunities to improve the selection process of genotypes of interest. Clonal planting material of at least six of these selections has been planted as large plots (100 plants per plot and two replications) to observe their performance before making a final selection of those showing economic potential to be released as fine or flavour varieties within the next 5-6 years.

The CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects were not isolated but complementary, allowing the continuity of research activities started before 1998, implementing new breeding activities during the period 1998-2003 and enlarging the scope of these activities in 2004-09 to embrace the inclusion of farmers in the process of evaluation and selection of new cocoa cultivars, all this as part of a participative research focus.

Current status of cocoa varieties

Unselected varieties

Most of the traditional cocoa fields in Ecuador are usually between 30 and 100 years old. They were established from seeds of pods harvested from trees selected by the producer. These selections were made on the basis of traits of economic importance such as a large number of healthy pods, preferably of large size, and resistance to diseases. In the past most farmers believed that these attributes would be transmitted to their descendants. They generally did not know about the phenomenon of genetic segregation preventing the replication in the descendants of the desirable attributes. Eventually only a few from the selected trees were productive.

Local observations showed that approximately 30% of the trees make up for 60 to 70% of the total production in a traditional cocoa field, while 30% of the least productive trees make up hardly for 6 to 8% of the total production. A few trees are able to produce 100 or more healthy pods per year, though in some cases these pods are small, an undesirable trait since it increases the time spent in harvesting. It has been observed that some trees do not produce any pods at all.

During the 1960s and 1970s INIAP distributed hybrid seed from a group of crosses among Nacional x Trinitario types selections and Upper Amazon genotypes (Scavina 6, Scavina 12, Silecia 1 and IMC 67, among others), showing at the time resistance to witches' broom. This planting material became part of the traditional cocoa fields, mainly in the central and northern cocoa-growing zones of the coastal region.

The cause for the gradual increase in the production, and hence exports, from the 1950s up to the present is the increase in the area planted to cocoa rather than higher yields. The accelerated planting of the clone CCN 51 is responsible for most of the new cocoa fields during the last decade, as well as for an important jump in the total production. However, a study by Amores (1999) concluded that a modest increase in productivity did take place in the second half of the 20th century as a result of technology transfer activities and release of hybrid seeds and clones by INIAP.

Recommended varieties

In the late 1970s a group of superior cocoa clones were released by INIAP for commercial planting. These were the output of a process that started in the late 1940s and early 1950s. After visiting hundreds of farms in distinct cocoa-growing zones, a number of trees were selected based on visual observations. The high-yielders with some disease resistance were identified and selected for further cloning. The clones were used to set up observation plots in the EET Pichilingue. Several years of careful evaluation led to the selection of the clones EET 103, EET 96, EET 95, EET 64, EET 48 and EET 19 to be released as clonal varieties of the Nacional type.

Observations completed in the framework of the CFC/ICCO/Bioversity project showed that the combined planting of clones EET 103, EET 96 and EET 95 yielded 15% higher than a mixture of all recommended clones. Based on this conclusion, more emphasis is placed on the multiplication and supply to farmers of these three clones.

Additional observations in the framework of the project allowed concluding that the clone EET 103 has a wide range of adaptation to several growing environments, ranking it as a sort of "universal clone". Finally, the experiences and formal information provided by both the CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects, and others conducted simultaneously, provided valuable inputs for the attempts related to the zonification for the planting of new cocoa fields, using the varieties recommended by INIAP.

Unfortunately, simultaneous comparison of these Nacional-type clones to CCN 51, a high-yielding clone lacking the alleles which are typical of the Nacional variety, as clearly demonstrated by several genetic analyses, showed that they yield quite less than CCN 51 under non-irrigated conditions. However, in spite of its good physical qualities (large size, homogeneity, etc.), CCN 51 is not graded as a fine or flavour variety on the market.

In the worst case CCN 51 yields twice as much as EET 103. However, under irrigation this difference is limited to only 20% in a couple of zones where comparative studies were completed. Moreover, the difference in yield between EET 103 and CCN 51 was not statistically different in a trial conducted in a "piedmont" area with good rainfall distribution; the altitude of the experimental site is 500 m above sea level and this location possibly has to do with the result.

In early 2009 a new group of cocoa clones was released for commercial planting, adding these up to the stock of Nacional-type clones INIAP is currently recommending. These clones are EET 575 and EET 576, adapted to the central part of Manabí, an important cocoa-growing zone with rainfall near to 1000 mm. The clones EET 544 and EET 558 are also part of the group of clones recently released. These are adapted to the zone of Chongon and related areas marked by a rainfall that is quite concentrated and ranging annually from 300 to 500 mm. This zone is a new frontier to grow cocoa under irrigation. Before their release, the new clones were compared simultaneously to CCN 51 under irrigation during the dry season for several years. The conclusion was that there were no statistical yield differences between these new Nacional-type clones and the CCN 51 variety.

These results clearly show that there are opportunities to select genotypes equipped with enough genetic potential for further development and release as fine or flavour cocoa varieties. When these were subjected to increasing levels of technical management, including irrigation and cultural disease control practices, they turned into highly productive cocoa fields. Hence the idea that all Nacional-type cocoa cultivars yield poorly is beginning to be scientifically questioned. Establishment of large demonstration plots and the availability of enough qualified planting material will help to improve the rate of adoption for these new fine or flavour cocoa varieties.

Multiplication of recommended varieties

Though additional efforts are required to demonstrate on a commercial scale the qualities of the new varieties, demand of qualified planting material is on the rise, largely fuelled by government programmes which are promoting the planting of cocoa of the Nacional type. The objective is to keep and reinforce the world presence of Ecuador as the main supplier of fine or flavour cocoa.

INIAP provides some 600 000 plants of Nacional-type clones annually. However, this is not sufficient to meet the current demand and has created a problem of transparency within the market of planting material. Several private nurseries that sell Nacional-type plants have emerged in the past few years. These nurseries have difficulties to demonstrate that the planting material is of the Nacional type. Besides, the plant marketing chain involves many intermediaries, which increases the problem and generates growing claims.

The search for alternatives to overcome this bottleneck is a pending task for INIAP. We are betting on the use of somatic embryogenesis technology to increase substantially the supply of qualified planting material of the Nacional type in future. In fact, after proving that the clonal plants originated by this technology perform much better (higher yield, precociousness, better growth pattern, etc.) than those produced through the traditional methods of cloning, we just recently established a clonal garden with these type of plants and plan to establish more gardens in the near future. Expectations are that we could be able to multiply by four the current INIAP's capacity to deliver clonal planting material to farmers.

In addition, a mechanism is also under discussion to authorize the functioning and monitoring of private nurseries to produce and offer qualified Nacional-type material, as long as these are duly supervised and certified by a government instance after checking through genetic profiles that the material being sold conforms to the genetic make-up of the recommended Nacional-type clones.

Farmer participatory selection activities

Farm surveys

Visiting cocoa farms is always an enriching experience as it offers opportunities to learn from visual observations and discussions with farmers about their perception on the yield variability of the trees that make up their cocoa fields, the factors that most limit yield from their particular perspective, and other issues. A survey was carried out to extract information and know the opinion of the cocoa farmers about selected agro-socio-economic issues influencing the traditional cocoa-producing process in their holdings.

Three distinct cocoa-growing zones were selected for the survey. Geological and geographic references were used to name the zones: (1) LLanura aluviales (floodplains), (2) Piedmont (slopes of the western part of the Andean range) and (3) Northern Esmeraldas (near to the Colombian border). These zones are responsible for two thirds of the total cocoa production in Ecuador. Thirty farmers were surveyed in each zone. The analysis of the information collected revealed differences in their socio-economic situation and in the technical challenges they face during the process of cocoa production.

The main conclusions of the survey are as follows:

1. Producers of the alluvial plain zone, technologically speaking, are ahead from the cocoa growers in other zones, as many of them use furrow irrigation, thus doubling or tripling the average productivity of the country. However, the farmers' average age is the highest (≥ 55 years).
2. The producers in the Piedmont zone have the highest literacy level (≥ 6 years at school) and showed the highest interest regarding the introduction of new varieties

and innovating management technology, including irrigation if high-yielding varieties are available.

3. The lowest average age corresponds to farmers in the zone of Northern Esmeraldas. They also showed great enthusiasm at the time of discussing about new management technologies to increase cocoa yield, though they also have the lowest literacy level.
4. In all zones most farmers (86%) share the idea that diseases represent the greatest limiting factor of cocoa yield, placing drought as the second limiting factor (40%).
5. In general, most farmers showed a genuine interest to be part of participative research processes to evaluate and identify improved cocoa varieties adapted to the zone where they live.

These conclusions represent valuable inputs to be taken into account at the moment of assigning resources (time, personnel, money, etc.) to be invested in research, technology transfer, training, credit programmes and other development tools to promote the production of fine or flavour cocoa varieties. Thus they stand out as an important output of the project, allowing a better viewing of the scenario and challenges faced by cocoa production in Ecuador.

Farm selections

The implementation of the CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects was fruitful regarding the gathering of new cocoa accessions that morphologically conform to the Nacional variety type. These collections enriched the EET Pichilingue germplasm bank, making it larger and hopefully richer in genetic diversity. Budsticks from the trees selected in farmers' fields were used to fix their genetic traits through clonal multiplication. Furthermore, the multiplied planting material was used to set up observation plots usually made up of 5 to 10 plants per plot.

These introductions represent genetic resources for future evaluation and identification of clones having a particular combination of traits justifying further development as commercial varieties. It is to be recalled that the clones currently recommended for commercial use by INIAP (EET 103, EET 96, EET 95, EET 48, EET 62, EET 19, EET 575, EET 576, EET 544 and EET 558) come from early selections made in traditional farmers' fields along different cocoa-growing zones.

This gathering activity was better planned and conducted during the CFC/ICCO/Bioversity project, as a key part of its central concept based on a participative research approach with the inclusion of producers to improve the process of selection of useful genotypes. A total of 12 farms were visited with the farmers in the three zones surveyed. The main output of this activity was the identification of 84 superior cocoa trees. Budsticks were gradually collected from these to obtain clones and thus fix their genetic attributes.

Northern Esmeraldas was the zone where the highest number of cocoa farms were visited and the highest number of trees identified for further cloning. The greater emphasis placed in this zone responded to our interest to find trees that exhibited phenotypic traits resembling Criollo cocoa types to widen the genetic basis of our germplasm bank and explore the possibility of developing in the long run a Criollo-type variety adapted to the Northern Esmeraldas zone. Trees showing the Criollo type have been frequently observed in earlier visits to this zone.

This objective was largely attained, representing another important output of the project. At the time of writing up this report, all the collected material is set up in the field and undergoing evaluation. It is expected that at the end of the evaluation process we will be able to select genotypes equipped with a particular combination of attributes to justify their further development as commercial fine and flavour cocoa varieties.

To complement this collecting activity of interesting Criollo types, a modest fraction of the funds allocated to the CFC/ICCO/Bioversity project was used in late 2008 to explore a relatively remote area of the Cayapas-Onzone river basin in the zone of Northern Esmeraldas. This was a joint cooperative search with another local project sharing the same objective. More than 300 accessions (clones and seeds) were gathered. The collected material was set up as observations plots made up of 5 to 10 plants at the EET Pichilingue. A partial duplicate is held by the University of Esmeraldas.

We can conclude that both projects, and particularly the CFC/ICCO/Bioversity project, have been generous in opportunities to search, identify and gather cocoa genotypes of interest in different farmers' fields. This is certainly another important output of the research efforts sheltered by this project, which contributed to widen the genetic variability of the germplasm bank held by INIAP for the genetic improvement of the crop. One of the objectives of the Northern Esmeraldas collection is in the long run to work towards the development of fine or flavour Criollo-type cocoa varieties adapted to this particular zone.

On-farm variety trials

All the 84 trees selected during the survey in different farmers' fields of the Alluvial Plains, Piedmont and Northern Esmeraldas zones were multiplied as clones and introduced into the EET Pichilingue as observation plots to study their performance. These introductions have enriched the genetic variability of the germplasm bank with the benefits it will bring for the future of the breeding of fine or flavour cocoa varieties in Ecuador. The next step will be a genetic characterization of these accessions to assess the extent of the variability that has been added to the bank and to develop their potential for use in breeding and selection.

Twenty-five of the eighty-four selected trees are participating as clonal treatments in farmers' field trials together with experimental clones of INIAP. These trials were established in the areas of Las Naves (Piedmont zone), Moraspungo (Piedmont zone), Milagro (Alluvial Plain zones), Simon Bolivar (Alluvial Plain zone), Colon Eloy (Northern Esmeraldas zone) and Muisne (South Esmeraldas zone) and are currently under evaluation.

Preliminary results from these trials, particularly those from Colon Eloy (Northern Esmeraldas) and Simon Bolivar (Alluvial Plains) look particularly promising for the future selection of genotypes adapted to these zones. Within the next 2 or 3 years we may be ready to make final selections to release cultivars that can be of commercial interest.

A formal experiment including all 25 clones which are participating in the distinct farmers' trials is currently conducted at the EET Pichilingue. The clone named 'Las Naves 8', coming from a tree selected in a farm of the Piedmont zone, stands out clearly from the others due to the large number of harvested pods per tree. Two large replicated plots are in the process of being planted with this clone to monitor its performance and that of other interesting cultivars set up in the same site. Some of these are clones of hybrid trees selected from hybrid families produced and planted during the CFC/ICCO/IPGRI project, but their evaluation was completed under the CFC/ICCO/Bioversity project.

On-station breeding efforts

Clone selection

Several clone comparison trials were established at the beginning of 1999 as part of the CFC/ICCO/IPGRI project. All were kept under evaluation until the first 3 years of the CFC/ICCO/Bioversity project. Research funding is always insufficient but we did find ways to stretch and complement the resources allocated by CFC with those of other sources. This way we kept these trials ongoing for a number of years until we had accumulated a sufficient amount of data to reach valid conclusions.

The different clonal variety trials were assigned the following names:
1. International Clone Trial
2. Local Clone Trial I
3. Local Clone Trial II
4. Observation plots for Nacional-type plants and their parental trees
5. Trial to compare populations of Nacional cocoa type under normal and high planting density
6. Study of the effect of the rootstock on the vigour of the scion.

Regarding the international clones introduced to evaluate their local performance and adaptation, none yielded higher than CCN 51 and EET 103 used as controls. The clone CCN 51 positioned itself far away from the rest with a yield almost double from that of EET 103, which came in second position. It is important to underline the low incidence of witches' broom symptoms (vegetative and cushion brooms) shown by CCN 51, demonstrating its good level of resistance to this disease. However, we cannot say the same regarding moniliasis, as observed on several instances. After corresponding lab and field tests, the clones IMC 47 and VENC-4-4 were classified as resistant to the "mal del machete" disease. The clone EET 59 showed a significant level of moniliasis resistance that should be exploited for breeding. All the clones in this trial were also characterized for sensorial traits.

The main conclusion based on the results of the Local Clone Trials points out that none of the commercial or experimental clones perform better than CCN 51 regarding yield or disease resistance. In the best case the yield difference was 70% in favour of CCN 51 and in the worst case this clone yielded several times higher than the lowest-yielding Nacional-type clones. The highest-yielding Nacional-type clones after CCN 51 were EET 103 and EET 575.

EET 575 was released as a commercial clone in 2009 due to its good adaptation in the province of Manabi, an important cocoa-growing zone. More importantly, its yield level is comparable to that of CCN 51 under irrigated conditions. This is supported by the results of a trial where CCN 51, EET 575 and other clones were tested simultaneously for a number of years. It should be noted at this time that CCN 51 performs better than any Nacional-type clone with no irrigation. It exhibits some drought resistance, possibly as an expression of an osmotic adaptation mechanism to this stress condition. Apparently, the Nacional-type clones do not show this physiological adaptation, thus being more susceptible to water stress.

On the other hand, the comparison of plants derived from seeds of pods harvested from selected trees identified in traditional cocoa fields with the clones of the same trees revealed that the seedlings always yielded higher than their clones. These results seem to provide additional evidence that clones derived from apparently superior trees are not a guarantee of good performance for reasons that we will not discuss here. However, the treatment made up of the mixture of clones EET 103, EET 95, EET 96 and EET 62 recommended by INIAP for commercial planting showed the highest yield compared to any other treatment, demonstrating why they are improved Nacional-type varieties.

The results of yet another trial suggested that yield is reduced by 50% when the planting density is doubled to 2200 plants per hectare as compared to the normal density (1100 plants/ha), possibly due to extreme resource competition aggravated by drought. This result is applicable also for other cocoa-growing areas. It would be interesting to know what will happen if intermediate density levels were tested. Would the yield increase or decrease? We need to clarify that the plants tested were derived from seeds.

The effect of pruning was also measured in the same study but the yield difference between pruned and unpruned plots showed no statistical significance, though pruning did cause a slight yield decrease. Since these are seed-derived plants, it is valid to ask what the response would be for clonal plants subjected to several levels of planting density and pruning (0, 25, 50 and 75% removal of the canopy). An additional pruning study concluded in 2008 with clonal materials clearly showed a gradual depressive effect on yield by increasing the intensity of this practice. A 25% pruning level did not reduce yield, suggesting that light pruning works fine for cocoa as compared to drastic pruning.

Another study to explore the differences among clones for the rates of flowering, setting of fruits, fruits affected by cherelle wilt, diseased pods and final dry bean weight was completed in 2009. Results showed significant statistical differences. CCN 51 produced three times more flowers than EET 103. However, the percentage of flowers setting fruits in the clone EET 103 was twice that of CCN 51. This result is in agreement with previous findings showing that the number of flowers does not represent a yield-limiting factor in cocoa production.

In the same study CCN 51 and EET 103 were the highest-yielding clones though CCN 51 yielded twice more than EET 103. Averaging through all clones we found that only 54 flowers per tree (i.e. 19% of all the flowers produced) were able to set fruits. Some 45% of the fruits were affected by cherelle wilt. Only 32% of the fruits developed and ripened normally up to harvest while 20% of them reached harvest time as diseased pods.

The effect of rootstock vigour on the growth and yield of the scion of two clones, EET 103 and CCN 51, was also studied. No clear differences in yield were detected in response to this factor for any of the two clones. However, it must be noted that the quality of the data was affected by excessive variability in the number of plants per plot since many did not survive. Replication of the trial to confirm these results is strongly suggested.

As described above, the project generated a large amount of information regarding several commercial and experimental clones, making an important contribution for a better understanding of the technical problems hindering cocoa productivity. These results have clearly opened avenues to build up new work hypotheses to guide future research efforts towards improving the agronomy and the genetic base of the cocoa crop.

Hybrid selection

As part of the workplan for the CFC/ICCO/IPGRI project, several trials to compare groups of hybrid families were established in 1998. These families originated from crossing schemes duly discussed, formulated and executed between the local cocoa team and the international Project Coordinator. A couple of trials that started earlier than 1998 were incorporated into the project workplan to be further evaluated. Most of the trials were kept under evaluation until 2008, well into the period of execution of the CFC/ICCO/Bioversity project.

Under the umbrella of the project, a last trial to compare hybrid families was planted in 2009, including as breeding parents the selections made in some experiments completed in the period 2007-08. The recombination of genetic traits associated to high yield, disease resistance and sensorial quality is the main objective. This trial will be conducted during the next 6 years to make additional selections of superior trees which will be further cloned.

Other activities started in early 2008 or even early 2010 under the umbrella of the CFC/ICCO/Bioversity project include the planting of two new experiments to compare groups of clones derived from superior trees selected in the distinct hybrid trials conducted and completed earlier. The objective of both trials is to select clones that best reflect the favourable traits of the trees they came from, and seem promising for further development as commercial varieties.

Although research funds are always scarce, we found ways to complement funding from different sources, including the CFC/ICCO/Bioversity project, to make progress in our breeding objectives. Maintenance and data registration for most of the trials that started during this project continued for a sufficient number of years to achieve results leading to valid final conclusions. The CFC/ICCO/Bioversity project provided a vital support to complete the evaluation and analysis.

The hybrid trials were defined as follows:

1. Comparison of 18 cocoa hybrid families of the Nacional type in the zone of Quevedo
2. Comparison of 21 cocoa hybrid families of the Nacional type in the zone of Quevedo
3. Comparison of 16 cocoa hybrid families obtained by crossing parents showing disease resistance and high-yielding traits
4. Comparison of 16 hybrid families obtained by crossing Nacional-type promising clones equipped with resistance to witches' broom
5. Comparison of 12 hybrid and selfed families obtained by crossing several Nacional-type clones, including CCN 51
6. Comparison of 20 hybrid families obtained by crossing selected Nacional-type cocoa clones showing disease resistance and high-yielding potential
7. Comparison of selfed cocoa progenies of clones that were used as parents in different crossing schemes
8. Comparison of non-inoculated and inoculated asymptomatic plants regarding witches' broom incidence and belonging to the same hybrid family
9. Comparison of hybrid families obtained by crossing homozygous clones of the Nacional variety with others showing a high degree of heterozygosity
10. Comparison of different hybrid families produced and sent by the countries participating in the project
11. Evaluation of the hybrid family (EET 183 x Pound 7) x UF 273 introduced from Costa Rica to participate in a study to analyse the stability of quantitative trait loci (QTLs)
12. Comparison of clones of hybrid trees selected for disease resistance and yield performance, Trial I
13. Comparison of clones of hybrid trees selected for disease resistance and yield performance, Trial II
14. Comparison of a group of clones planted in large replicated plots (100 plants) of trees selected for disease resistance and high-yielding traits.

Some of the trials were established recently (2008-10) and have not yet generated results. Sensorial evaluation data of pulp and cotyledon of many hybrid trees was also carried out to test the hypothesis that flavour quality traits can be predicted from testing of the pulp and possibly also from the cotyledon. The flavour variability of the fresh beans for the trees tested and sensorial traits measured is surprisingly large.

A number of selections were made from the different trials completed during the span of the CFC/ICCO/Bioversity project. They include those originated in families of the following crosses:

CCAT 2119 x CCAT 4668	EET 445 x CCN 51	EET 451 x EET 387	CCN 51 x EET 387
EET 451 x EET 387	EET 426 x EET 387	CCN 51 x EET 387	EET 416 x EET 534
CCN 51 x CCAT 2119	EET 446 x EET 547	CCN 51 x EET 233	EET 559 x CCN 51
EET 445 x EET 400	EET 578 x EET 547	CCN 51 x EET 416	EET 575 x EET 462
EET 416 x EET 400	EET 48 x EET 95	CCN 51 x EET 233	

As stated earlier, most of these selections have already been cloned to participate in new clone comparison trials established in the period 2008-10. It is expected that within the next 5 or 6 years these trials will be the source of new fine or flavour cocoa varieties. Since these are being conducted at the EET Pichilingue, the first beneficiaries will be the cocoa producers of the zone of Quevedo which provides one third of the total cocoa exports.

Lessons learned

Finding the right balance among the different resources (funds, trained people, time, land, etc.) that take part in any research process is certainly a difficult task. We should recognize that this was a great challenge, which we could not always meet. Talking about new avenues and pieces of research was easier than putting them into practice given the scarcity of resources available. Financial shortage was only one of the constraints; others were the insufficient level of training of the people taking part in the project and the limited availability of required expertise and of staff time.

In Ecuador the students preparing an agronomy degree must complete and defend a piece of research. Therefore we recruited students who had finished their university studies to manage the different trials and record the data to be used as the basis of their research report (thesis). The students needed training and close supervision that demanded an important amount of the time of the few senior researchers available before they were able to show some level of autonomy. Most of the time there were other cocoa research projects on quality, agronomy and technology transfer going on at the same time, putting a lot of pressure on the time resource provided by scientists with some experience. Sometimes there was no time to share.

Administrative issues and staff transfers constituted other obstacles. In short, several challenges were confronted but most of them were finally overcome. We learned that reaching an adequate balance for research resources was difficult but both the CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects provided us with opportunities to gain valuable experience. We should also recognize an important weakness: lack of publications in scientific journals.

Conclusions

Impact of the CFC/ICCO/Bioversity project

Both the CFC/ICCO/IPGRI and CFC/ICCO/Bioversity projects were key factors to re-launch cocoa breeding research in Ecuador, which had been discontinued since the mid-1970s.

New germplasm collections, breeding populations and clone selections obtained during this international collaboration effort represent a strong platform for future release of genetically improved varieties for the benefit of cocoa producers. These project outputs articulate nicely into a strategic plan under preparation by INIAP to guide the development of superior varieties, placing emphasis on fine and flavour cocoas, for the next 25 years.

It is important to note that some selections made during the CFC/ICCO/Bioversity project are already being tested in main cocoa-growing regions. Besides, six clones multiplied from selected best hybrid trees have been established recently (early 2010) and replicated as 100-plants-plots at the EET Pichilingue. Demonstration and future release of at least a couple of fine or flavour cocoa varieties is the main objective.

Training of local people in scientific, technical and conceptual skills, to visualize, formulate, conduct and evaluate breeding and selection processes, also represent a valuable output derived from both projects. In this context, three of the several students who conducted research in the framework of both projects were recruited as INIAP staff. Others work as cocoa agronomists in the private sector. In addition, the experience acquired through the different project activities has become an important asset for the Cocoa Programme staff when they are solicited to advise or participate in the formulation of other local cocoa projects regarding research and technology transfer, as well as to take part in their execution.

Perspectives

The outputs from both projects (collected germplasm, enrichment of variability, hybrid populations, selection of best hybrid trees, availability of promising clones, farmers' field trials, staff experience, international links, etc.) are being built into a strategic plan under development by INIAP, aiming at gradually producing and releasing genetically improved fine or flavour cocoa varieties for the next 25 years, as well as improving the associated agronomical practices. It is expected that irrigation studies on cocoa will become an important line of research to develop most of the potential of future high-yielding cultivars – indeed almost half of the producers who answered the survey conducted in three distinct cocoa-growing zones mentioned drought as a limiting factor, following cocoa diseases which come first.

A field day will take place within 6 months to show advances in somatic embryogenesis technology and set up the first clonal garden to promote an alternative method of clonal multiplication. This will hopefully help to get a substantial increase in the supply of qualified planting material for the production of fine or flavour cocoa. In the medium term this technology may become an important tool to distribute genetically improved cocoa varieties for commercial planting.

A cocoa field day is planned to take place within a year from now to release two new Nacional-type clones with "Arriba" flavour. These will be recommended for the south-eastern part of the Guayas river basin, an important cocoa-growing zone.

It is also expected that within 3 years a couple of additional Nacional-type clones, products of the CFC/ICCO/Bioversity/INIAP project, will be added to the recommended commercial cultivars for the same zone.

Within a similar period of 3 years we expect to release new varieties for the zone of Northern Esmeraldas. Within 6 years a cocoa field day will take place to release new commercial fine or flavour cocoa varieties adapted to the central (Quevedo zone) and northern parts of the Guayas river basin, which together produce 40% of the total cocoa exports. Within 12 years from now we may be ready to release new fine or flavour cocoa flavour varieties adapted to the Northern Ecuadorian Amazonia, an expanding border for this crop. We plan to multiply and send selections of this project to the Amazonian region in the near future.

Meanwhile the breeding work will continue to keep enriching the hybrid population base for selection and development of new cocoa varieties. In this line of thought an F2 hybrid population was produced in 2009 and a formal trial was set up early in 2010 for evaluation. It is expected to start backcrossing to increase the frequency of desirable alleles in selected hybrid trees in the near future.

The contribution to the increased capacity of the local cocoa research team to set up a work basis and formulate a vision to develop this resource base into improved fine or flavour cocoa varieties, accompanied by the best agronomy practices, is by itself the most important project output.

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Selection of new varieties on-farm and on-station in Ghana

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Introduction

Cocoa production constraints

When farmers are interviewed, they deplore that cocoa production in Ghana is saddled with low yields and poor establishment. Many factors are involved, among which the inherent low yields of available varieties, high disease incidence of *Phytophthora* pod rot (Ppr) and cocoa swollen shoot virus (CSSV) diseases, high pest incidence and gradual degradation of the environment, including soils and climate changes. Thus, in the development of improved cocoa planting materials, these factors are those to be considered. In the past, varieties have been developed to confront these problems and successes have been achieved. However, more efforts are needed to develop varieties that can significantly outperform those that are currently available. Average yields in Ghana are around 500 kg/ha. Several germplasm materials have been assembled at the Cocoa Research Institute of Ghana (CRIG) but few are utilized in the development of new varieties.

In this project the approach was two-fold: (1) to evaluate both parents in old trials and their progenies at both laboratory and field levels for the following traits: ease of establishment, early yielding, high yielding, resistance to prevailing diseases and pests, and quality; (2) to evaluate both the germplasm already collected and that recently introduced for the above traits. The ultimate objective is to select useful parents for the development of elite varieties for the Ghanaian farmer.

Cocoa breeding objectives

Cocoa breeding objectives in Ghana have focused on high yield, early bearing, good establishment ability, disease and pest resistance and good quality of beans.

Current status of cocoa varieties

Unselected varieties

Before the establishment of seed gardens, farmers in Ghana were using unselected varieties from their farms as planting materials. Even though this practice has decreased as a result of the establishment of seed gardens, some farmers still use unselected varieties for various reasons.

Recommended varieties

Many more farmers now use recommended varieties which are mainly Upper Amazon crosses.

Multiplication of recommended varieties

This is done by the Seed Production Unit in close collaboration with CRIG, using vegetative propagation methods (budding) for the parent materials and manual pollinations to produce in monoclonal seed gardens the F1 progenies which are then supplied to farmers.

Farmer participatory selection activities

Farm surveys

A total of 1500 farmers were interviewed across all six cocoa-growing regions of Ghana. The results showed that over 60% of farmers are more than 45 years of age and over 40% of the farms are old (over 25 years). In 2004 the average yield in farms was low (435 kg/ha) and the incidence of black pod (95%) was much higher than that of CSSV (32%). Farmers are aware of the type, variation and potentials of cocoa trees in their farms. Three main types of planting materials were encountered: Amelonado (26%), Upper Amazon (69%) and Series II hybrids (48%). Farmers are good at identifying best traits in their farms and use these among other criteria to select the best planting materials.

The high levels of black pod disease indicate that varieties improved by CRIG succumb to the disease, hence the need to breed for resistant varieties. The significant replacement of Series II hybrids by Upper Amazon, and high incidence of black pod and low incidence of CSSV in farms show that varieties given to farmers appear to be resistant to CSSV and prone to black pod caused by *Phytophthora megakarya.* Most of these varieties released to farmers were developed for resistance to CSSV, not to *P. megakarya*. This confirms the impact of breeders' selections on farmers' plantations. Farmers' indigenous knowledge in cocoa farming could be tapped by researchers through Farmer Participatory Research to breed for suitable planting materials for farmers.

Farm selections

Survey of farms and interviews with farmers were used to select trees with high agronomic potential for the study. Selection was based on farmers' knowledge of trees with high numbers of pods and low disease incidence. Budwood from farmers' farms was subsequently collected in the six cocoa-growing regions in the country. These clones were budded onto mixed Upper Amazon rootstock. One hundred and forty clones selected from farms in 2005 were planted at CRIG in a replicated field trial of 2 ha in 2006. Missing trees were replaced. The repeated replacement of trees created difficulties in yield data collection as trees in the trial have different ages.

On-farm trials

Forty-two farmers were initially selected for the on-farm trials in the Ashanti and Western Regions. Each farmer was requested to prepare an acre of land. Open-pollinated seedlings of the best trees selected by farmers, seedlings from the seed gardens and breeders' materials were used as test plants. Only 31 farmers' plots could be planted, representing 31 acres.

Girth and mortality data were taken each year, beginning one year after planting. Analysis of the results showed that breeders' selections performed significantly better than farmers' selections in terms of vigour in both regions. Although there was no significant difference in mortality between the treatments, breeders' materials tended to have a better survival rate. By June 2008, evaluation of the plots revealed that only seven farms in the Western Region and eight in the Ashanti Region met the minimum set standard for continued monitoring and data analyses. Data on yield could not be taken before termination of field monitoring in June 2009.

On-station breeding efforts

Clone selection

Three clone evaluation trials were conducted: the Local Clone Observation Trial, the Local Clone Selection Trial and the International Clone Trial.

- **Local Clone Observation Trial**

The trial was established to evaluate the parents in the breeding programme in the field for yield and resistance to diseases. The initial attempts to establish the trial in 2004 was not successful. The trial was re-established in 2006 with 98 clones. Girth measured 22 months after transplanting showed significant differences among the clones with T 90/1383 recording the largest girth of 44.4 cm. Across genetic groups, the Trinidad (T) clones had the largest stem girth, whereas the Scavina (SCA) and Parinari (PA) groups had the smallest stem girth. Two clones selected from Goaso (in the Ashanti Region of Ghana) recorded average girth smaller than that of the Scavina group.

- **Local Clone Selection Trial**

The objective of this trial was to evaluate selected local clones as candidates for commercial production with emphasis on yield, resistance to black pod and CSSV diseases, and for resistance to capsid damage. The trial, however, did not establish well and not much useful data could be obtained. The 25 clones including controls were screened for CSSV resistance. Mean scores for CSSV resistance were based on the percentage of budlings that showed symptoms of the disease after inoculation. T 65/326 and T 65/238 were the clones with the lowest infection scores for CSSV.

- **International Clone Trial**

The objective was to evaluate selected international clones for yield and for resistance to black pod, CSSV and capsids. Data analysis on yields from 2005 to 2009 showed that bean yield was highest in clone EET 59 (1755.90 kg/ha). Compared with the standard clones (T 85/799 and T 79/501), some clones have shown considerable promise for bean yield and resistance to black pod. Mean scores for CSSV resistance were based on percentage of budlings that showed symptoms of the disease after inoculation. Clones with high scores were more susceptible to the CSSV disease. Out of the 25 selections screened, only BE 10 was significantly more infected than the mean of the other clones.

Hybrid selection

- **The Regional Variety Trial**

Eighteen progenies were tested in the Regional Variety Trial. Stem diameter taken at 30 months after planting and mortality of test plants showed that the trial established poorly. Only PA 13 × PA 19 and T 60/887 × ICS 89 had more than 70% of stands remaining as at 2009. Other test genotypes had less than half of stands remaining. Data collection on this trial was terminated.

- **Determination of combining abilities for yield and black pod resistance of selected clones**

This experiment was established as a 6 × 4 factorial trial and a 6-parent diallel trial. In the factorial experiment, the female parents included PA 7, PA 150, NA 33, IMC 76, T 60/887 and T 63/971. The male parents were SCA 9, SCA 6, IMC 53 and IMC 67. The six female parents were used for the diallel experiment.

The results are based on data obtained in 2006-07, 2007-08 and 2008-09. Among the female clones tested in the factorial trial, PA 7 and T 60/887 had the best combining abilities for dry bean yield and resistance to black pod disease. SCA 9 had the best combining ability for yield and black pod incidence among the male clones tested. The best crosses were obtained between SCA 9 and female clones NA 33, T 60/887, PA 7 and T 63/971.

In the diallel trial, PA 150 and T 60/887 had the best combining abilities for dry bean yield and black pod infection. Reciprocal differences were evident in the performance of the progenies for both yield and resistance to black pod disease.

The screening for CSSV resistance did not reveal any clear pattern for general combining abilities of the clones used. Specific crosses may be more important. The best crosses include NA 33 × IMC 76, NA 33 × T 63/971, IMC 67 × IMC 53 and IMC 76 × PA 150. The least resistant crosses were PA 150 × NA 33, PA 7 × SCA 9 and NA 33 × PA 7.

Population breeding

- **Population breeding trials**

Five trials were conducted under the population breeding effort, aiming at estimating the value of four of the Upper Amazon cocoa genetic groups for breeding. These populations include the Parinari (PA), Nanay (NA), Iquitos (IMC) and Trinidad (T) clones. This involved the following progenies in separate experiments:

1. Selfed clones
2. Inter-group crosses
3. Intra-group crosses
4. Inter- and intra-group crosses compared
5. The 29th progeny trial area (PTA) consisting of selfed, inter- and intra-group crosses.

- **Progenies of selfed clones**

Twenty-four clones consisting of six clones in each of the four genetic groups were evaluated. Data obtained from 2004-05 to 2008-09 were analysed. Significant differences ($P<0.05$) were observed between progenies for dry bean yield, yield component traits and black pod incidence. Averaged across years, the differences between progenies for dry bean yield were slight. For the cumulative yield over the period of the experiment, the highest yields were recorded for NA 79; progenies of four other clones (T 85/799, PA 107, T 79/501 and NA 34) had yields that were not significantly different. The lowest bean yields were recorded predominantly among progenies of selfed IMC clones. The pod rot damage ranged from 13.1 to 26.6%. Three of the highest-yielding progenies (PA 107, NA 79 and T 79/501) were among those with the highest incidence of black pod disease. However, no significant correlation was observed between the two traits ($r=0.155$, $P=0.469$). Between genetic groups, bean weight per pod and average bean weight were highest in the IMC progenies and least in the Parinari and Nanay selfed progenies. Differences between genetic groups for dry bean yield and incidence of black pod disease showed an inverse pattern. Based on genetic groups, no significant differences were observed between the selfed progenies of the Parinari, Nanay and Trinidad clones for bean yield or black pod incidence. IMC progenies had significantly lower dry bean yields; however, incidence of black pod disease was significantly lower among these progenies than in those of the other genetic groups.

- **Progenies of inter-group crosses**

This experiment evaluated 105 progenies obtained from test crosses of clones belonging to the four genetic groups. Yield records were obtained in 2006-07, 2007-08 and 2008-09. The highest bean yields were obtained in the 2006-07 period, after which the high incidence of black pod disease reduced yields. Comparing the genetic groups when used as females, no differences were observed in bean yield between progenies. Similarly, for a particular genetic group, the progenies obtained from crossing with each of three testers performed the same for bean yield irrespective of the tester. These observations held true for the 2007-08 and 2008-09 seasons. For the black pod incidence, however, the PA and IMC genetic groups recorded the lowest scores when crossed with NA 33. Differences in black pod incidence were not observed between testers for the T and NA genetic groups.

In 2006-07, the best progenies for bean yield for the tester NA 33 were obtained with T 79/501, PA 121 and PA 150. For tester PA 7, best progenies were obtained with IMC 45 and IMC 105; for tester IMC 76, with PA 134 and T 16/613; and for tester T 16/613, with PA 121 and PA 134.

The data for the 2007-08 and 2008-09 seasons were analysed to obtain the combining abilities of the clones for yield. The number of pods produced per plot was used as index for yield. For the IMC clones, only IMC 45 consistently performed well for pod production. Among the NA clones, none showed consistency in contributing positively to pod production or bean yield over the 3-year period. PA 121, PA 134 and PA 150 displayed good combining abilities for pod production. Among the T clones, only T 79/501 showed consistent positive contribution to yield. The best specific crosses include PA 121 × T 16/613, PA 150 × NA 33, T 79/501 × NA 33, PA 134 × T 16/613 and PA 121 × NA 33. This holds true for both pod production and incidence of black pod disease.

- **Progenies of intra-group crosses**

Progenies of crosses within the PA, NA, IMC and T genetic groups were evaluated for bean yield and resistance to black pod disease. Data obtained over 3 years (2006-07, 2007-08 and 2008-09) were analysed. Between genetic groups, yield was lowest among the Parinari genetic group; however, the differences were not significant in any of the 3 years. Incidence of black pod disease generally increased each year from the 2006-07 season to the 2008-09 season. Differences between genetic groups for incidence of black pod disease were not statistically significant in any of the 3 years.

Within the Nanay group, the best progenies for yield and resistance to black pod disease were obtained from NA 242 x NA 535, NA 421 x NA 535 and NA 33 x NA 34. Progenies obtained from intra-PA crosses did not show consistent ranking for yield or black pod incidence. Progenies obtained from the cross PA 7 x PA 150 appeared to be among the best progenies for yield in each year. The level of black pod incidence recorded on this progeny was however high. Progenies from two crosses, IMC 39 x IMC 44 and IMC 49 x IMC 68 were outstanding for yield among the intra-IMC crosses. For the progenies obtained from crosses within the T genetic group, none was outstanding over the 3-year period for yield or resistance to black pod disease.

- **Progenies of inter-group and intra-group crosses compared**

Twenty-five progenies of inter-group crosses and 16 progenies of intra-group crosses of the four Upper Amazon cocoa genetic groups were evaluated in this trial. Data obtained from 2005-06 to 2008-09 on yield and black pod incidence are reported. In each of these years, there were no significant differences between the progenies of inter-group and intra-group crosses for bean yield and resistance to black pod infection. In each year, however, the intra-group crosses had a higher value for dry bean yield than the inter-group crosses. Over the 4-year period, the best progenies of the inter-group crosses included PA 150 x IMC 23, NA 34 x PA 67 and PA 150 x T 63/967. The best progenies of the intra-group crosses included PA 150 x PA 151 and PA 303 x PA 67.

- **Progenies of the 29th PTA consisting of selfed, inter- and intra-group crosses**

This experiment is located at Apedwa in the Eastern Region of Ghana. The trial consists of 30 Upper Amazon cocoa progenies comprising 3 selfed progenies, 5 intra-group and 22 inter-group crosses. The best progenies for yield include PA 150 x PA 300, PA 134 x T 16/613, PA 134 x NA 33 and T 16/613 x NA 33. Among these, PA 150 x PA 300 was the least infected by black pod disease whereas PA 134 x T 16/613 recorded the highest incidence of the disease.

- **Recurrent selection for identification of superior clones**

The experiment was set up to evaluate the performance of progenies of selected parents as candidates for clone selection with emphasis on resistance to black pod and CSSV diseases. Twenty-five progenies of various crosses were investigated. Data obtained from 2004-05 to 2008-09 were analysed following a repeated measurement procedure to identify the best progenies over the first 5 years. The analyses revealed that four progenies consistently had high yields in each year, leading to high average yields per year: PA 7 x PA 150, PA 150 x Pound 7, T 65/326 x T 60/887 and Pound 10 x Pound 15.

Lessons learned

- Farmers' indigenous knowledge is very useful in farmer participatory research.
- Participatory approach to selection and breeding is necessary.
- Good initial establishment of trials is required to produce good data and results.

Conclusions

- Materials selected by breeders give better performance in terms of initial establishment than unselected materials.
- Many interesting crosses were selected for further use in breeding.
- Intra-group crosses yielded as well as inter-group crosses.

Perspectives

Such breeding programmes should not be terminated abruptly. Provision should be made for the programme to continue till conclusions can be drawn

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Farmer participatory and collaborative approaches to cocoa breeding in Malaysia

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Introduction

Cocoa is the third commodity of economic importance in Malaysia, contributing to more than RM 3 billion in export earnings. The cocoa hectarage and production have consolidated from the declining trend that occurred in the 1990s. As of 2009, the cocoa area was estimated to be 20 561 ha with a total production of 18 152 tonnes (Malaysian Cocoa Monitor 2009). The smallholder sector contributes 86.6% of the hectarage, of which 38% in Sabah, 33% Sarawak and 26% in Peninsular Malaysia.

Cocoa production constraints

Several factors affect the cocoa production, including pest and disease infestation, competition with other commodities and increasing agricultural input prices. Cocoa pod borer (CPB), vascular streak dieback (VSD) and black pod (BP) diseases have been the more problematic pest and diseases but they are manageable with good agricultural practices. Competition with other agricultural commodities is mainly for land use, while increases in costs of agricultural inputs have increased the production cost.

Cocoa breeding objectives

High yields have been the basic objectives of our breeding programmes, but other objectives that include breeding for resistance to common pests and diseases, bean characteristics, flavour, desirable agronomic traits, etc. are incorporated to produce superior planting materials. Other basic studies such as inheritance, sexual compatibility and assisted molecular breeding are also part of the breeding objectives.

Current status of cocoa varieties

The planting materials used in the country evolved from hybrids in the 1960s up to the early 1990s to clonal planting materials in recent years. Most of the cocoa areas are planted with recommended clones.

Unselected varieties

Most of the clones used by the cocoa growers are recommended clones and unselected varieties are rarely used. Generally, three to five clones are recommended for planting in locations of similar agro-climatic conditions.

Recommended varieties

While hybrids had been widely planted in the 1960s to 1980s, clonal materials have been the most widely planted in recent years. The Malaysian Cocoa Board (MCB) recommended several clones for planting that include, among others, MCB C1–9, PBC 123, KKM 22, KKM 1 and BR 25. Recommendation of clones is based on the agro-climatic conditions and disease type and severity in the areas.

Multiplication of recommended varieties

Hybrid seeds are produced from the polyclonal seed gardens and they are open-pollinated. On the other hand, clones are grafted on suitable rootstock. Grafting methods used are bud-grafting and twig-grafting (side- and top-grafting). The age of rootstock used depends on the scion, i.e. green for a juvenile budding and semi-brown for patch/bud grafting.

Farmer participatory selection activities

During the first year (June 2004–June 2005) 85 smallholders were surveyed and 93 individual trees were selected. Selection criteria included pod bearing, disease incidence, visual vigour and feedbacks from the farmers visited. However, out of the 93 clones selected 62 were finally established at the MCB R&D centres for further evaluation in the observation plots. The individual trees selected were from Sabah (23), Peninsular Malaysia (32) and Sarawak (7).

Farmers' selection

Two approaches were used in the farmers' selection: field evaluation by the researchers and preference of the farmers. The researchers' selections on the farmers' areas were from the hybrid populations, while the farmers' selections were those they had found to be outstanding in terms of pod production. Among the farmers' selections, CFC 2-27 (Changkat Lada, set 1), CFC 2-73 (Tenom and Ranggu), CFC 2-71 (Taiping) and CFC 2-75 (Changkat Lada, set 2) were the more outstanding clones in the respective locations.

On-farm trials

Ten multilocation on-farm trials (MLFT) were established; they were located at Tawau (2 trials), Ranau (1), Tenom (1) in Sabah; Changkat Lada (2), Taiping (1), Jerantut (1), Air Hitam (1) in Peninsular Malaysia and Serian (1) in Sarawak.

There were 20 clones in each site: 9 breeders' selections, 9 farmers' selections and 2 local control clones. Each plot had 10 trees (2 x 5) spaced at 3 m x 3 m; thus each trial site is 0.5 ha.

Among the farmers' selections in each sites, the highest-yielding clones were CFC 2-27 (Changkat Lada, set 1), CFC 2-57 (Changkat Lada, set 2), CFC 2-14 (Taiping), CFC 2-47 (Air Hitam), CFC 2-16 (Wakuba), CFC 2-61 (Serian and Tenom), CFC 2-9 (Ranau) and CFC 2-18 (Ranggu). CFC 2-72 was the superior clone in Serian, Air Hitam, Ranggu and Ranau, CFC 2-73 in Tenom and Ranau, CFC 2-71 in Taiping and CFC 2-75 in Changkat Lada, set 2. Generally the control clone CFC 2-63 produced more pods than the other clones in all sites. With regard to VSD, the tolerant clones were CFC 2-37, 40, 50 (Changkat Lada, set 2), CFC 2-2, 06, 61 (Wakuba), CFC 41, 56, 57, 61, 62 (Serian) and CFC 48, 61 (Tenom).

On-station breeding efforts

Clone selection

Clone selection was conducted on the three progeny trials established under the previous CFC/ICCO/IPGRI project. A total of 50 individual trees were selected and are being evaluated in a clone trial at the MCB R&D Centres in Sabah. Sixteen individual trees from the previous progeny trials were selected based on their low incidence of CPB and they are evaluated in clone trials at MCB R&D Centres in Madai and Hilir Perak.

Three Farmers' Selection Observational Plots (FSOPs) were also established at the MCB R&D Centres at Madai, Jengka and Hilir Perak. Sixty-two clones are included in each site plus three controls (KKM 22, BR 25 and PBC 123). There are 12 trees in each unreplicated plot, with the exception of the controls that are replicated 5 times.

The top-yielding clones in the FSOPs were CFC 2-50 (in Madai and Jengka) and CFC 2-36 (Hilir Perak).

The tolerance level of clones to VSD varied in their respective location. In Madai Station, CFC 2-24, 25, 33, 36, 37, 41, 42, 43, 44, 46, 49, 50, 60, 61 and 62 showed the same tolerance level as PBC 123 (control) with no symptom of VSD infestation recorded. At Jengka, CFC 2-10 and CFC 2-58 showed lower VSD score (0.00) than the control clone PBC 123 (0.50). In Hilir Perak, CFC 2-52 and CFC 2-56 showed the same level of VSD tolerance as the control clone PBC 123 with no symptoms of VSD infestation.

The mean dry bean weight (BW), bean number per pod (BNP) and pod value (PV) of the clones of the FSOP at Madai were 1.07 g, 34 and 30, respectively. While 57% of the clones had BW over 1.0 g, only 5% had PV below 25. Clones CFC2-9, 2-1, 2-18, 2-29, 2-55 and 2-65 were among those with desirable BW (over 1.10 g) and PV (below 25).

The mean BW, BNP and PV of the clones at Jengka were 1.12 g, 37 and 25, respectively. Clones CFC 2-3, 2-5, 2-18, 2-28, 2-53, 2-63A and 2-64B had BW and PV over 1.10 g and below 25, respectively.

Hybrid selection

Data recording on yield, pest and disease incidence and pod/bean characteristics were continued for the progeny trials from the previous project. There were 3 progeny trials consisting of 25 progenies in set I, 16 progenies in set II and 20 progenies in set III. In set I, Desa 1 x IMC 67 was the most prolific progeny, while progenies with Desa 1 parental material generally had lower VSD incidence than the others. Desa 1 x PBC 156 produced the highest yield in set II while PBC 123 x UF 676 exhibited lowest VSD incidence. In set III, KKM 22 x T 85/799 was the top yielder and progenies with Desa 1 as maternal parent had lower VSD scores than the others.

Although superior progenies were identified from the three progeny trials, they were not intended to be recommended for commercial planting. Elite individual trees that had high yield, good tolerance to pest and disease and superior bean qualities were selected for further clonal evaluation.

Population breeding

Three progeny trials were conducted in the previous project. The first set was meant to produce sibling populations that are high-yielding and tolerant to VSD; set II was meant for yield and tolerance to BP, and set III for yield and siblings of high yield and small height of the trees. Fifty individual trees were selected from the three trials, while sixteen were selected for CPB tolerance.

Lessons learned

Genetic material

The project enabled us to obtain new genetic material to be used in our future breeding programmes. New individual trees had been selected in the first project and these materials are potentially to be used as recommended planting materials.

Participatory approach in selection of individual trees

The farmers valued the individual trees they had selected; this created a sense of belonging and an eagerness to be involved in the project. Moreover, the farmers involved improved their knowledge on cocoa through technology transfer from the researcher.

Utilization of the selected clones

The on-farm trials have been terminated and the respective farmers are managing the plots. We had duplicated the farmers' selection in our Research Centres and will continue to evaluate the performance of the clones in the trials.

Conclusion – Impact of the CFC/ICCO/Bioversity Project

Cocoa genetic collection

The project had recommended genetic material that was indicated, among others, to be tolerant to BP, witches' broom and monilia, and good-yielding. We have collected most of this genetic material to be used in our breeding programme that includes, among others, breeding for tolerance to BP, preventive breeding and population breeding. In addition to the individual clone, the project enabled us to identify elite individual trees that exhibited high yield, tolerance to VSD, BP and other desirable traits.

Evaluation of potential clones

Sixty clones were selected from the farm survey and 50 individual trees were identified from the progeny trial for further clonal evaluation. In addition, 16 individual trees were identified for CPB tolerance based on morphological traits, crop loss and sclerotic layer hardness. The clones derived from the farmers' plots were evaluated in on-station and on-farm clonal trials. These clones will be further evaluated and constitute potential or candidate clones to be recommended for planting.

Capacity building

Capacity building was made possible in the project through exchange of visits between regional researchers in MCB and the Cocoa and Coconut Institute (CCI), Papua New Guinea. The project's implementation, progress and findings were discussed. Beside the regional meeting, information was also exchanged, among others, through project workshop, visit by the Project's Coordinator and publications.

Local breeding programmes

The projects have complemented and to a certain extent expedited the local breeding activities. We managed to increase our own and locally selected material through the project. While there is an increase in the genetic materials, new clones were derived from the farmers' survey and progeny trials. Potential clones have been identified to be used by the farmers.

Participatory breeding

Most of the farmers involved in the on-farm trial were enthusiastic about the trial and contributed to the project implementation and to its success. Although the size of each trial was 0.5 ha, the farmers were pleased to obtain good yield from it. The Common Fund for Commodities (CFC), previously unknown to the farmers, was widely publicized by those involved in the project.

Perspectives

Evaluation of the introduced and locally selected clones, on-station trials (progeny trials, local observation plots, farmers' selections and clonal trials) will be maintained and collection of data should proceed as scheduled. Superior introduced clones will be used as parental clones in the new progeny trials. Outstanding clones from the farmers' selections and those selected from the previous progeny trials will be evaluated in multilocation trials and some will be recommended for planting.

The on-farm trial was an opportunity to evaluate the breeders' selection in multi-environmental conditions and their suitability according to the locality. While the farmers' selections will enable us to identify new superior clones in the respective localities, it is unfortunate that we could not proceed to collect data after the project completion. The ten on-farm trials are distant from our station and travelling for monitoring consumed a large proportion of the component's budget. The on-farm trials had to be returned to the respective farmers.

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Selection of new cocoa (*Theobroma cacao* L.) varieties in on-station and on-farm trials in Nigeria

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Abstract

One of the major objectives of cocoa breeding research is the development of high-yielding cocoa genotypes (hybrids and/or clones) adapted to local growing conditions that can give optimum returns on investment to farmers. On-station and on-farm field evaluation were carried out in the CFC/ICCO/Bioversity project including the 1999 Hybrid Trial, Local Clone Trial, Regional Variety Trial, On-farm Participatory Hybrid Trial and Ex-Uhonmora Clones Observation Trial. From results obtained over a 10-year evaluation period, some hybrid families with significantly ($P<0.05$) greater productivity than the F3 Amazon control were identified. Some outstanding clones were also identified from the Local Clone Trial and Ex-Uhonmora Clone Observation Trial. On-farm trial of selected hybrids also confirmed the stability of precocity and high yields observed in the tested materials. In conclusion, in the framework of the CFC/ICCO/Bioversity project, a number of interesting hybrids and clones that can be fed into the formal cocoa seed production system have been selected. These have huge potential for improving cocoa productivity and income at the farm level as well as the national production output.

Introduction

The use of selected and improved planting materials with desirable traits in terms of high yield and disease and pest resistance is one of the key elements of a sustainable cocoa production system. Prominent among factors limiting efficient cocoa production in Nigeria are the unavailability of high-yielding varieties, old age of trees and plantations, black pod disease caused by *Phytophthora megakarya*, mirid attack caused by *Sahlbergella singularis* and the cocoa swollen shoot virus disease. Abiotic stress (drought) due to long and severe dry season stretching from December to April also poses a serious threat to cocoa production (Aikpokpodion 2007). The use of unselected planting materials has continued among farmers with little use of recommended improved planting materials (Eskes 2000). In the early 1990s, before the inception of the CFC/ICCO/Bioversity project, cocoa breeding effort was at its lowest due to poor government funding which has not significantly changed to date. There was therefore little or no research effort to select improved planting materials. The main objectives of the project were therefore to: (1) select high-yielding and early-bearing hybrid families and clones, (2) select individual progenies and clones showing a high level of resistance to *Phytophthora* pod rot, and (3) test selected crosses on-farm and evaluate their performance under farmers' production conditions.

Materials and method

On-station trials

The growing conditions of the on-station trials (Headquarters of the Cocoa Research Institute of Nigeria (CRIN), Ibadan) were as follows: no shade (only plantain shade during the first 3 years), no fertilizer or pesticide application system, on a deforested sandy loam alfisol in a marginal cocoa-growing area in a derived savannah zone with about 1250 mm annual rainfall. The trials were irrigated during the dry seasons of the first 3 years after establishment.

- **1999 Hybrid Trial**

This trial consisted of 23 bi-parental crosses and open-pollinated F3 Amazon control. These were planted at the CRIN Headquarters in Ibadan (07.02°N, 03.09°E; 122 m above sea level) in June-July 1999. The trial was laid out in a randomized complete block design with 6 replications consisting of a 10-tree plot each, planted at 3 m x 3 m spacing. Observations were made on: precocity (earliness in bearing), estimated as pod production during 24–36 months of field establishment; tree jorquette height (cm), girth (cm); canopy size (1 to 5); canopy shape (1 to 5); number of pods/tree; number of beans/pod; bean length (cm); bean width (cm); fresh bean weight (g); dry bean weight (g); nib weight (de-shelled bean weight in g); shell percentage (%); pod index; tree productivity per hectare and butter fat content (%). Data collection was made over a 10-year period from 2000 to 2009.

- **Regional Variety Trial**

In the period 2005-06, the Regional Variety Trial comprising 24 hybrids, 9 crosses from Ghana, 4 from Côte d'Ivoire, 3 from Cameroon, 2 common crosses from Nigeria and 6 local control crosses was established in a randomized complete block design with 6 replicates. Each hybrid genotype consisted of a 10-tree plot per replicate planted at 3 m x 3 m spacing. The growing condition was a medium-shade (plantain shade + indigenous tree species scattered around the plot). Observation was made on precocity (earliness in bearing) estimated as pod production within 24–36 months of field establishment, tree jorquette height (cm), girth (cm), canopy size (1 to 5), canopy shape (1 to 5) and number of pods/tree. Records were taken from 2007 to 2010.

- **Ex-Uhonmora Intra-Nanay and Intra-Parinari clones**

Twenty-five individual genotypes selected from the Uhonmora introduction plot of the 1967 Trinidad Introduction consisting of Intra-Nanay and Intra-Parinari crosses were selected and established at the CRIN Headquarters in Ibadan in 2000. These were planted out in a completely randomized design at a spacing of 3 m x 2.5 m with a variable number of trees per clone. Although field establishment commenced in 2000, it was not completed until 2002. After the clone trial had been successfully established on the field, an evaluation of yield in terms of annual pod production was carried out between 2007 and 2009 when the grafted trees were fully established.

- **Local Clone Trial**

In this trial, 16 local clones which were parents of the 23 bi-parental progenies tested in the Hybrid Trial were established in a randomized complete block design with 6 replicates between 2000 and 2002. Each clone was planted in a 10-tree plot per replicate and planted at 3 m x 3 m spacing. Observations were made on quantitative traits including number of pods/tree, number of beans/pod, bean length (cm), bean width (cm), fresh bean weight (g), dry bean weight (g), nib (de-shelled bean) weight (g), shell percentage (%), pod index, tree productivity per unit hectare and butter fat content (%).

- **On-farm Trial**

In 2006, 19 of the 25 selected farms in the 3 cocoa-producing regions of Nigeria were planted with 8 to 11 hybrids (Table 1) selected from the 1999 Hybrid Trial in an on-farm trial using farmer participatory approaches. These selected materials were the breeders' selection to be compared with farmers' best materials. Ten stands per genotype were planted at a spacing of 3 m x 3 m. Farmers carried out cultural operations such as weeding and irrigation during the dry season. Data was collected on jorquette height (cm), girth (cm), canopy size, canopy shape, number of branches and number of pods/tree between 2007 and 2009.

Table 1. Selected hybrid genotypes tested in the On-farm Trial across 19 farms in Nigeria

Identification codes	Genotypes
A1 x L1	T 67/7 x T 22/28
A1 x M1	T 65/7 x N 38
A1 x N1	T 65/7 x T 9/15
A1 x O1	T 65/7 x T 101/15
B1 x M1	T 101/15 x N 38
C1 x L1	T 86/2 x T 22/28
C1 x N1	T 86/2 x T 9/15
C1 x P1	T 86/2 x T 16/17
C1 x R1	T 86/2 x T 57/22
D1 x Q1	T 82/27 x T 12/11
G1 x T1	C 77 x C 67

Results and discussion

- **1999 Hybrid Trial**

Analysis of variance (ANOVA) showed highly significant ($P<0.001$) main and interaction effects of genotype, season and year on pod production (number of pods per tree) and on crop productivity (dry bean yield in kg/ha) among all the 23 hybrids and F3 Amazon genotypes tested (Tables 2a and 2b). Mean annual productivity for all 24 tested genotypes over 9 harvest seasons is presented in Figure 1. Mean separation presented in Tables 3a and 3b also showed that pod production and crop productivity among the 24 genotypes tested differed significantly. While pod production was high among genotypes 20, 9, 7, 16, and 13 compared to the F3 Amazon control, it was low in others, e.g. genotypes 15, 18 and 23. The mean annual dry bean yield (Table 4) multiplied almost 5-fold over the 9-year period from 338.46 kg in the first year of harvest (2001-02 season), that is 2 years after field establishment, to 1560.53 kg/ha in the eighth season (2008-2009). From the table, we observe that in the first year of harvest, 2 years after field establishment in 1999, all the hybrids except the F3 Amazon control made yields ranging from 39 kg/ha for T 82/27 x T 16/17 to 544 kg/ha for T 101/5 x N 38. Six hybrids produced between 440 and 544 kg/ha. In contrast, the F3 Amazon control did not produce until after 3 years of field establishment. This evidence of precocity showed superiority of some of the hybrids over the F3 Amazon control.

Table 2a. ANOVA for annual pod production of 23 bi-parental hybrids and F3 Amazon control over eight seasons from 2001 to 2009

Source	DF	SS	Mean square	F Value	P>F
Genotype	23	8765.43	381	2.59	<0.0001
Season	1	24178.87	24178	164.46	<0.0001
Year	7	221869.36	31695	215.59	<0.0001
Genotype*Season	23	5376.42	233	1.59	0.0363
Year*Genotype	158	35448.73	224	1.53	<0.0001
Year*Season	7	58541.70	8363	56.88	<0.0001
Year*Genotype*Season	150	32155.87	214	1.46	0.0002
Error	11271	1657058.07	147		

Table 2b. ANOVA for annual crop productivity (kg/ha dry bean yield) of 23 bi-parental hybrids and F3 Amazon control over eight seasons from 2001 to 2009

Source	DF	Mean square	F value	P>F
Genotype	23	953665	3.57	<0.0001
Year	7	51355038	192.26	<0.0001
Season	1	109959997	411.67	<0.0001
Genotype*Year	158	413021	1.55	<0.0001
Genotype*Season	23	569193	2.13	0.0013
Year*Season	7	19194406	71.86	<0.0001
Genotype*Year*Season	150	369791	1.38	0.0014
Error	11271	267107		
Total	**11640**			

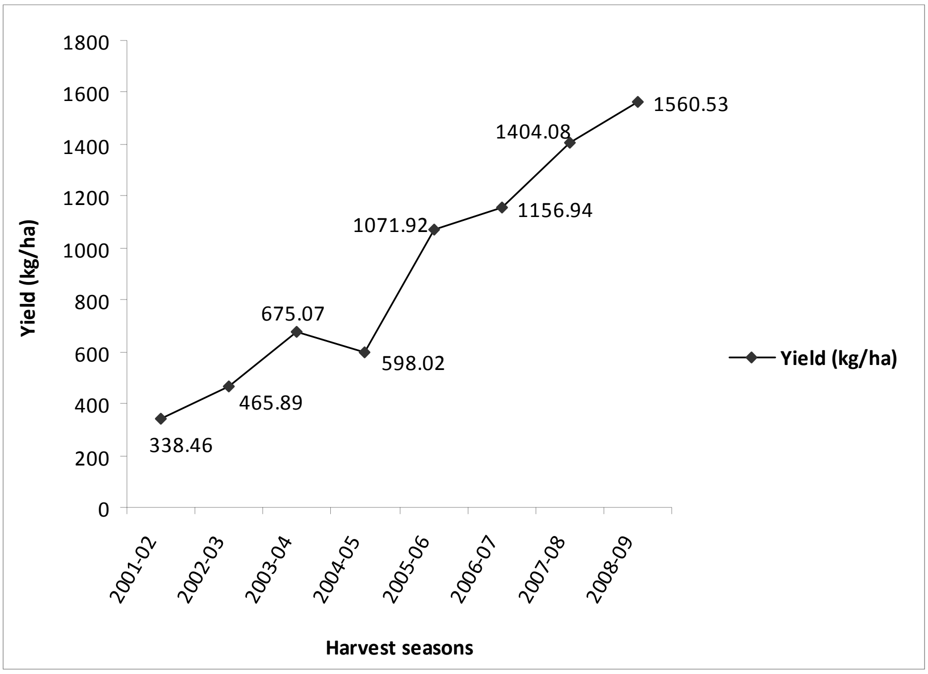

Figure 1. Mean dry bean yield (kg/ha) gradient of 23 bi-parental hybrids and F3 Amazon control showing almost 5-fold increase in the eighth harvest season (2008-09) compared to the first harvest in 2001-02.

Table 3a. Student-Newman-Keuls test for number of pods produced per tree of 23 hybrid genotypes and F3 Amazon control over eight seasons from 2001-02 to 2008-09

Genotype	N[1]	Mean	SNK grouping[2]						
T 86/2 x T 53/8	293	16.68					a		
T 65/7 x N 38	660	16.35				b	a		
T 65/7 x T 57/22	600	15.69				b	a	c	
T 53/5 x T 12/11	582	15.55			b	d	a	c	
T 82/27 x T 12/11	556	15.45			b	d	a	c	
P 7 x PA 150	497	15.42			b	d	a	c	
T 9/15 x T 57/22	467	15.37			b	d	a	c	
F3 Amazon control	**436**	**15.26**		**e**	**b**	**d**	**a**	**c**	
T 12/11 x N 38	551	14.98		e	b	d	a	c	
T 86/2 x T 57/22	377	14.52		e	b	d	a	c	f
T 86/2 x T 65/35	364	14.30		e	b	d	g	c	f
T 53/5 x N 38	672	14.04	e	b	d		g	c	f
T 101/15 x N 38	507	13.99	e	b	d		g	c	f
T 65/7 x T 101/15	574	13.77	e	b	d		g	c	f
T 86/2 x T 9/15	532	13.45	e		d	h	g	c	f
PA 150 x T 60/887	554	13.44	e		d	h	g	c	f
T 65/7 x T 22/28	413	12.88	e		d	h	g		f
P 7 x T 60/887	535	12.84	e		d	h	g		f
T 65/35 x T 30/13	367	12.71	e			h	g		f
T 86/2 x T 16/17	396	12.20				h	g		f
T 65/7 x T 9/15	507	12.05				h	g		f
T 86/2 x T 22/28	301	11.84				h	g		
T 65/7 x T 53/8	433	11.67				h	g		
T 82/27 x T 16/17	467	11.08				h			

1 N = sample size
2 Means not followed by the same letters are significantly different at 5% level of probability.

Table 3b. Student-Newman-Keuls separation of average crop productivity (kg/ha dry bean yield) obtained over eight seasons (2001–09) in 23 hybrid genotypes and F3 Amazon control

Genotype	N[1]	Mean	SNK grouping[2]						
T 65/7 x N 38	660	710.69				a			
T 101/15 x N 38	507	678.08		b		a			
P 7 x PA 150	497	673.16		b		a			
T 86/2 x T 53/8	293	652.97		b		a			
T 12/11 x N 38	551	637.33		b		a			
F3 Amazon control	**436**	**625.19**		**b**		**a**		**c**	
T 86/2 x T 57/22	377	618.93		b	d	a		c	
T 53/5 x N 38	672	609.84	e	b	d	a		c	
T 82/27 x T 12/11	556	595.56	e	b	d			c	
T 53/5 x T 12/11	582	585.19	e	b	d	f		c	
T 65/7 x T 22/28	413	579.24	e	b	d	f		c	g
T 65/7 x T 57/22	600	573.88	e	b	d	f	h	c	g
P 7 x T 60/887	535	525.19	e	i	d	f	h	c	g
T 86/2 x T 9/15	532	520.61	e	i	d	f	h	c	g
T 9/15 x T 57/22	467	518.14	e	i	d	f	h	c	g
T 65/7 x T 101/15	574	516.65	e	i	d	f	h	c	g
PA 150 x T 60/887	554	515.49	e	i	d	f	h	c	g
T 86/2 x T 65/35	364	515.16	e	i	d	f	h	c	g
T 86/2 x T 16/17	396	510.40	e	i	d	f	h		g
T 82/27 x T 16/17	467	499.92	e	i		f	h		g
T 86/2 x T 22/28	301	481.95		i		f	h		g
T 65/7 x T 53/8	433	468.25		i			h		g
T 65/35 x T 30/13	367	465.16		i			h		
T 65/7 x T 9/15	507	456.44		i					

1 N = sample size
2 Means not followed by the same letters are significantly different at 5% level of probability.

Table 4. Dry bean yield (kg/ha) of 23 bi-parental hybrid crosses and F3 Amazon control in 2001-02 and 2008-09 seasons. Main crop (August to January) and light crop (February to July).

Genotype	First production year (2001-02)			10th year of establishment (2008-09)			CSA[1] (cm^2) 2008-09	HE[2] (kg/ha/cm^2) 2008-09
	Main crop	Light crop	Total annual	Main crop	Light crop	Total annual		
T 65/7 x T 22/28	368.62	71.58	440.2	1177.35	424.34	1601.69	132.50	15.11
T 12/11 x N 38	47.47	51.99	99.46	1246.73	381.50	1628.23	157.45	10.34
T 65/7 x T 9/15	233.64	82.44	316.08	1020.10	322.57	1342.67	132.56	10.13
PA150 x T60/887	256.62	103.00	359.62	1105.96	421.81	1527.77	137.92	11.08
P 7 x T 60/887	39.01	109.69	148.7	1039.16	338.42	1377.58	147.68	9.33
P 7 x PA 150	152.94	33.94	186.88	1386.67	461.62	1848.29	113.52	16.28
T 65/7 x T 57/22	61.63	70.04	131.67	1188.35	446.37	1634.72	130.17	12.56
T 53/5 x N 38	40.59	206.56	247.15	1189.54	466.95	1656.49	164.60	10.06
T 65/7 x N 38	515.83	0.00	515.83	1470.71	571.52	2042.23	161.34	12.66
T 53/5 x T 12/11	232.14	90.25	322.39	1124.29	515.90	1640.19	145.06	11.31
T 65/35 x T 30/13	242.48	94.67	337.15	914.66	323.32	1237.98	98.21	12.61
T 86/2 x T 9/15	194.76	273.71	468.47	951.06	356.74	1307.80	157.37	8.31
T 9/15 x T 57/22	369.12	0.00	369.12	887.73	256.88	1144.61	133.80	8.56
F3 Amazon (control)	0.00	0.00	0.00	1116.64	403.65	1520.29	88.49	17.18
T 86/2 x T 22/28	219.73	252.58	472.31	513.55	458.85	972.40	104.92	9.27
T 82/27 x T 12/11	45.57	123.74	169.31	1220.47	462.49	1682.49	136.31	12.34
T 86/2 x T 16/17	106.23	67.73	173.96	836.44	606.01	1442.45	121.34	11.88
T 65/7 x T 53/8	51.28	0.00	51.28	834.39	563.32	1397.71	120.98	11.55
T 65/7 x T 101/15	368.62	153.11	521.73	980.33	619.79	1600.12	131.67	12.15
T 86/2 x T 53/8	47.47	0.00	47.47	1136.59	444.97	1581.56	96.06	16.46
T 86/2 x T 65/35	233.64	0.00	233.64	932.57	550.82	1483.39	107.28	13.83
T 101/5 x N 38	256.62	287.69	544.31	1427.87	690.46	2118.33	122.77	17.26
T 82/27 x T 16/17	39.01	0.00	39.01	1095.76	637.17	1732.93	125.50	13.81
T 86/2 x T 57/22	152.94	66.28	219.22	1003.05	654.12	1657.17	152.74	10.85
Grand mean	188.78	149.68	338.46	1087.58	472.95	1560.53		
Standard error	27.33	19.07		23.07	8.49			

[1] CSA = Cross-sectional area of the tree trunk
[2] HE = Harvest efficiency

From further analysis of the 23 hybrids in terms of productivity over the 10 years of assessment, 12 out of the hybrids outperformed the F3 Amazon control. Some of the hybrids including T 65/7 x N 38, T 101/15 x N 38 and P 7 x PA 150 reached 1 t/ha in 5 years of establishment (2004-05) at the third harvest season and produced more than 2 t/ha in the 2007-08 season, 8 years after establishment. This is in contrast to F3 Amazon which yielded 1 t/ha in the sixth year and has not reached the 2 t/ha mark even after 10 years of field establishment. Assessment of the mean productivity over the eight seasons indicated that the F3 Amazon control, though with a lower mean yield (626 kg/ha), did not differ significantly from the five highest yielders (637–711 kg/ka). This also indicated the potentials of the earlier released variety in terms of yield in a well-managed field. However, some of the poor yielding hybrids such as T 86/2 x T 22/28 did not produce 1 t/ha even after 10 years of field establishment.

The harvest efficiency (HE) measured as crop productivity per cross-sectional area of the tree trunk (kg/ha/cm^2) of each hybrid genotype (Table 4) and related to tree vigour was evaluated and classified into three groups, namely, High (14.1–18.0), Medium (11.1–14.0) and Low (8.0-11.0). Results obtained showed that hybrid genotypes T 101/15 x N 38, P 7 x PA 150, T 65/7 x T 22/28 and T 86/2 x T 53/8 with the F3 Amazon were in the High harvest efficiency group. Eleven hybrid genotypes were in the Medium HE, while eight others were in the Low HE group.

A further evaluation of other important variables including overall crop productivity, physical bean quality, resistance levels against *Phytophthora* and mirid attacks and flavour analysis will determine the final selection of these high-yielding genotypes as commercial varieties.

- **Regional Variety Trial**

As at 2009, survival percentage ranged from 38.3% for T 85/799 x T 79/501 (Nigeria Local control) to 76.7% for A1/154 x T60/178 (Ghana). Analysis of variance (ANOVA) showed a highly significant ($P<0.001$) effect of genotype on the jorquette height and girth. This year effect was also significant ($P<0.001$) on jorquette height, girth and number of branches per tree. However, there was no significant genotype x year effect on jorquette height (cm), girth (cm), canopy shape and number of branches. Results showed that the stem girth was greatest in PA 13 x P 19 (12.8 cm) and least in T 85/799 x T 79/501 (7.8 cm) (Table 5). The jorquette height was also greatest in PA 13 x P 19 (134.3 cm), followed by SNK 12 x PA 150 (128.7 cm), and shortest in T 85/799 x T 79/501 (92.0 cm). Assessment of juvenile yield showed that the mean number of pods produced per tree ranged from 9.7 to 18.5 in 2008, the first year of production in the high class comprising SCA 24 x SNK 614, T 60/178 x T 85/87, A 1/154 x T 60/178, IFC 303 x PA 121 and F3 Amazon local control. In 2009, during the second year, the mean number of pods produced also ranged from 9.7 to 22.0 in (P 7 x PA 150) x IMC 47, T 85/799 x PA 120, PA 4 x P 7, T 65/7 x T 79/501, SCA 24 x SNK 614, T 60/178 x T 85/87, A 1/154 x T 60/178, IFC 303 x PA 121 and F3 Amazon local control. On the other hand, PA 13 x P 19 and T 85/185 x T 60/178 produced no fruit at all.

Table 5. SNK grouping showing differences in girth among 24 hybrids of the Regional Variety Trial in Nigeria

Genotype	Mean	SNK grouping
PA 13 x P 19	12.8	a
UPA 134 x SNK 64	12.7	a
SNK 12 x PA 150	12.6	ab
SCA 6 x (DR 1 x DR 38) x PA 120	12.6	ab
A 1/154 x T 60/178	12.5	ab
GU 144 x EQX 3338	12.4	ab
SCA 24 x SNK 614	12.0	abc
T 60/178 x T 85/87	11.6	abcd
T 85/799 x PA 120	11.4	abcd
(P 7 x PA 150) x AMAZ 15-15	11.4	abcd
T 65/7 x T 79/501	11.3	abcd
IFC 303 x PA 121	11.2	abcd
T 63/967 x T 17/524	11.1	abcd
GU 147H x NA 33	11.0	abcd
PA 4 x P 7	10.7	abcd
T 60/887 x SNK 413	10.4	abcde
T 60/887 x ICS 89	10.3	abcde
MAN 15-2 x T 85/799	10.2	bcde
T 65/7 x T 101/15	9.7	cdef
F3 Amazon (control)	**9.6**	**cdef**
(P 7 x PA 150) x IMC 47	9.6	cdef
A 1/154 x T 85/185	9.2	def
T 85/185 x T 60/178	8.2	ef
T 85/799 x T 79/501	7.8	f

- **Ex-Uhonmora Intra-Nanay and Intra-Parinari Clones**

A summary of pod production among the clones showed that in 2007, the mean pod production, 12.1 pods/tree ranged from 4.6 pods/tree for PA 187 x PA 70 to 29.6 pods/tree for NA 737 x NA 739. In 2009, the mean pod production, 29.1 pods/tree ranged from 10.3 pods/tree in NA 327 x NA 387 to 57.0 pods/tree in PA 184 x PA 70 and 56.6 pods/tree in NA 387 x NA 332. Analysis of variance showed significant ($P<0.001$) difference in pod production among the clones. Separation of mean (Table 6) carried out showed the following clones as the top eight best yielders with a mean range of 25.8–54.0 pods/tree: PA 184 x PA 70, NA 387 x NA 332, NA 737 x NA 739, PA 202 x PA 310, NA 387 x NA 20, NA 61 x NA 132, NA 26 x NA 387 and PA 73 x PA 6. This is in contrast to the poor-yielding ones such as NA 739 x NA 268, PA 67 x PA 310, NA 737 x NA 137, PA 187 x PA 70, PA 187 x PA 295 and NA 327 x NA 387 which yielded between 10.0 and 15.5 pods/tree between 2007 and 2009.

Table 6. Mean pod production from 2007 to 2009 in 25 Ex-Uhonmora clones in an observation plot at CRIN Headquarters in Ibadan

Genotype	N	Mean	SNK grouping
PA 184 x PA 70	9	54.0	a
NA 387 x NA 332	24	38.8	b
NA 737 x NA 739	15	36.9	bc
PA 202 x PA 310	15	30.0	bcd
NA 387 x NA 20	30	29.6	bcd
NA 61 x NA 132	18	27.6	bcd
NA 26 x NA 387	9	26.3	bcd
PA 73 x PA 61	4	25.8	bcd
NA 342 x NA 387	102	24.8	bcd
NA 173 x NA 387	3	23.0	bcd
NA 222 x NA 387	67	22.7	bcd
PA 293 x PA 295	12	22.6	bcd
PA 189 x PA 70	105	22.0	bcd
NA 295 x NA 69	48	20.7	bcd
NA 534 x NA 69	19	20.4	bcd
NA 184 x NA 70	25	19.5	bcd
NA 739 x NA 113	18	19.4	bcd
PA 168 x PA 295	65	19.0	bcd
NA 737 x NA 332	24	18.3	bcd
NA 739 x NA 268	8	15.5	cd
PA 67 x PA 310	6	15.3	cd
NA 737 x NA 137	28	14.1	cd
PA 187 x PA 70	15	11.8	d
PA 187 x PA 295	11	11.5	d
NA 327 x NA 387	12	10.0	d

N = number of samples

- **Local Clone Trial**

Analysis of variance showed significant ($P<0.001$) among the clones in number of pods produced per tree. The number of pods produced per tree in 2007 varied between 1.0 for T101/15 and 8.8 for T 82/27. In 2009, the mean number of pods produced per tree ranged from 3.2 for T 101/15 to 29.0 pods for T 16/17.

Conclusions

From the various trials conducted on-station, a number of interesting hybrids and clones have been selected as new cultivars to be released to the farmers while some other trials are still under evaluation. From the 1999 Hybrid Trial, at least 12 hybrids with superior performance over the F3 Amazon control have been identified. Results from on-farm evaluation of some of these hybrids confirm the stability of their performance in terms of precocity and superior yield potentials over local controls. These materials are to be officially released and fed into the formal cocoa seed production system in Nigeria. It is expected that use of these materials will greatly improve productivity and revolutionize cocoa production in Nigeria. A number of interesting clones and previously unutilized materials in the germplasm such as the Ex-Uhonmora materials were also identified. These clones would prove valuable for farmers who would be interested in growing cocoa clones in Nigeria.

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On-farm and on-station trials implemented in Papua New Guinea

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Abstract

The Papua New Guinea Cocoa and Coconut Institute (CCI) participated in the two components of the CFC/ICCO/Bioversity project *"Cocoa Productivity and Quality Improvement: a Participatory Approach"* (2004-2010). Several activities including two sets of on-farm trials in East New Britain and Madang, together with two on-station trials of advanced testing and combining ability testing of farmers' Trinitario clones were included under Component 1. Component 2 of the project included two advanced hybrid and hybrid clone trials including continuation of some activities from the previous CFC/ICCO/IPGRI project *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* (1998-2003). Activities in both components of the project were well integrated into the general activities of CCI's cocoa breeding programme.

Most of the project activities in Tavilo, East New Britain province, started before the beginning of the project in March 2004. Accordingly, data collection in those activities also started earlier. Two new activities, Advanced Trial of selected clones from the Local Clones Observation Trial (LCOT) and New Experimental Hybrids were successfully established in Tavilo after 2004 as well as on-farm activities in Madang. Data collection in all project activities initiated after 2004 were not completed after the fifth year of the project. Additional data collection will be required for some time in order to assess the benefits of the activities. Despite the insufficient timing, some useful information on yield, vascular streak disease (VSD), black pod and other traits were collected. Both components of the project constituted a total area of 66.5 hectares including activities in Madang.

An event which significantly impacted on the project activities was the discovery of the cocoa pod borer (CPB) and its eradication operation in 2006-07. The CPB operations resulted in the delay of activities in Tavilo including loss of planting material, yield and other relevant data for various project activities. Prior to the arrival of CPB, most activities in Tavilo were progressing well. In most cases, the implementation of proposed activities was either on or ahead of schedule. Recovery from the CPB operations was slow in terms of implementation of activities. Activity 1.2 (on-farm testing) was the major activity of the project involving on-farm testing in East New Britain and Madang. Included in this activity were potential clones for near release to farmers in Papua New Guinea. Release of selected clones from this activity was delayed to obtain further information on the clones' reaction to CPB.

Preliminary screening for CPB tolerance was included in some project activities and is continuing. Not all materials were screened to date due to the large number of clones. This will be continued further after the project ending. Current results indicate a large majority of clones to be susceptible to CPB.

Introduction

Cocoa was first introduced into Papua New Guinea (PNG) at the beginning of the 20th century by German planters who own several large plantation estates in the country. Since then, cocoa has grown to be an important economic cash crop, fetching close to

US$100 million in export earnings for the country. This places cocoa as the third most important commodity cash crop after oil palm and coffee. Currently cocoa production in PNG is based on two germplasm origins, Trinitario (T) from the German introduction and Upper Amazon (UA) introduced in the 1960s. Two hybrid mixtures, SG1 and SG2 were developed from selected crosses between T and UA in the 1980s. The hybrids form the base for the majority of planting material grown throughout the country.

Several problems including yield variability, early yield decline and *Phytophthora* pod rot (Ppr) were identified with the hybrids. This resulted in the phasing off of the SG1 hybrid in 1986 and in the modification of the SG2 hybrid in 1994. To address the variability problems experienced with the hybrids, the Cocoa and Coconut Institute (CCI) embarked on a new breeding strategy in 1994, emphasizing the development of hybrid-derived clones. The aim is to capture the genetic uniformity in clones for yield, disease resistance and production stability. Apart from that, new hybrids are continually being developed and tested by the Institute, mostly for release to less advanced and isolated farmers.

Several hybrid and hybrid clone trials were established by the breeding section of CCI since 1995. The breeding trials include several trials established and maintained during the first and second projects funded by the Common Fund for Commodities (CFC): *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* and *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*. Both projects' activities were well integrated into activities and objectives of the breeding programme. The breeding section was able to improve and increase the scope of work in terms of establishing new experiments and managing those already existing with the financial support from the CFC. Under the second CFC project, the breeding section was able to establish and maintain ten different trials. Two trials were included as continuation from the first CFC project. The second CFC project comprised two components with experiments occupying a total area of 66.5 ha (Table 1).

Table 1. Experiments carried out in PNG during the second CFC project

Project component	Activity	Activity title	Trial size (ha)	Planting date	Location
1	1.2.a	On-Farm Trials in East New Britain	22.4	2000	ENBP*
1	1.2.b	On-Farm Trials Madang	9.6	2005-06	Madang
1	1.3.1	Advance Testing of Trinitario clones collected from farmers' fields	2.5	2003	ENBP
1	1.3.2	Combining Ability Testing of Trinitario clones with Upper Amazon	10.5	2000	ENBP
2	2.1.1.a	International and Local Clones Trial	3.2	1999	ENBP
2	2.1.1.b	International Clone Trial	2.8	2000	Madang
2	2.1.1.c	Local Clones Observation Trial (LCOT)	3.6	1998	ENBP
2	2.1.1.d	Modified Hybrid Trial	3.9	1999	ENBP
2	2.1.2.a	Advance Trial of selected LCOT clones	2.2	2007	ENBP
2	2.1.2.b	New Experimental Hybrids	5.8	2007	ENBP
		Total area (ha)	**66.5**		

* ENBP = East New Britain Province

Analysis of results

Only results of trials that are advanced enough to deliver conclusions will be presented here.

On-farm trials

- **Multilocation testing of clones in East New Britain Province**

Multilocation testing is the final step in the breeding and testing of new cocoa planting material before final release to farmers. Its purpose is to test the material for adaptability to different locations and environments in order to identify possible genotype x environment (G x E) interaction. Management levels are also an important component of the environment. Depending on the sites and on who is involved, multilocation testing encourages farmer participation in the final selection of new cocoa planting material under their management systems. A two-way learning process where the farmer learns from the researchers and researchers learn from the farmers is enhanced through multilocation testing.

CCI shifted its emphasis from hybrids to development of hybrid-derived clones in 1994. Since then it has continued to develop and test new clones. Prior to the release of the big and small Hybrid Clones (HC1-B and HC1-S) in March 2003, a new set of clones was selected for multilocation testing in 2000. The clones were tested in various sites in East New Britain and Madang under activity 1.2 of the CFC project. Selected clones under this activity were expected to be released in 2008-09. Release was postponed to enable further information on the reaction of the clones to cocoa pod borer (CPB).

Material and methods

Thirty-eight new and high-yielding experimental clones from an advanced trial of clones derived from crosses between T and UA were tested. In addition to these 38 clones, 6 provisionally released clones were used as controls. Based on the potential vigour, the clones were divided into four sub-groups: "big" (B), "intermediate" (I), "small" (S) and "very small" (VS). Each group was planted at an estimated optimal density of 625, 714, 833 and 1000 trees/ha respectively. Planting was done in 2000 with one replication per site with two rows per clone and per site for the four sub-groups. Sub-groups "big" and "small" had nine clones while "intermediate" and "very small" had ten clones each. Eight testing sites were located in East New Britain: CCI plantations (Tavilo, Kevera, Londip and Raulawat); Newmark plantations (Notremal, Stockholm and Tokiala) and National Agricultural Research Institute (NARI) plantation. Another testing site was in Stewart Research Station, Madang. Several from the 38 clones tested in East New Britain were also tested in on-farm trials in Madang.

Observations were done on yield (dry bean weight/ha), yield components (pod and bean weight), vigour (stem circumference), yield efficiency (YE = yield/stem section), vascular streak dieback (VSD) incidence, CPB severity and *Phytophthora* pod rot (Ppr) resistance (natural infection and detached pod test).

Results

Total yield variations among the four sub-groups tested during 2003–09 varied between 5891 kg/ha (very small) and 5156 kg/ha (intermediate). The highest yields came from the very small clones, followed by small clones (5353 kg/ha), big clones (5248 kg/ha) and intermediate clones (Tables 2 to 5). The relative yield for the big, intermediate and small clones in relation to the very small clones (highest yielders) was 89%, 85% and 92.6%. The average yield differences among the clones were directly related to the planting densities of the four sub-groups.

Among the big clones (Table 2), the three clones B2, B7 and B3 stood out as the highest-yielding during the period 2003-09; they were followed by the control clone TA 103. The relative yields in relation to the highest-yielding control for the three high-yielding clones were 130%, 116.1% and 100.1% for B2, B7 and B3 respectively. Among the three highest-yielding clones, B2 is highly susceptible to CPB and Ppr but highly resistant to VSD. Clones B3 and B7 are highly susceptible to CPB and moderately resistant to Ppr and VSD. Compatibility studies also showed that clones B2 and B7 are self-incompatible while B3 is partially self-compatible.

Among the intermediate clones, the control TA 203 was the highest yielder (Table 3). TA 203 is highly susceptible to CPB and Ppr but resistant to VSD. Clones I10 and I9 are tolerant to CPB while I2 is susceptible. All three clones are resistant to Ppr and VSD. Other clones with high tolerance to CPB among the intermediate clones are I1, I4 and I7. The majority of the intermediate clones are partially self-compatible except for three clones (I6, I7 and TA 203) which were self-incompatible.

Table 2. Total average dry bean yield, yield components, vigour and yield efficiency of big clones in the on-farm testing in six commercial plantations during 2003-08

Clone	Code	Pod weight (g)	Wet bean (%)	Dry bean yield (kg/ha)	Yield index[1] (%)	Stem section (cm^2)	YE[2] (kg/cm^2)
B2	12-4-4	335	35.4	8237.3	130.4	301.7	5.4
B7	77-4-6	505	33.1	7331.0	116.1	245.6	6.0
B3	22-3-5	330	24.1	6324.0	100.1	211.2	5.9
TA 103 (C)	37-13/1	900	24.5	6315.4	100.0	287.7	4.4
B1	12-2-12	310	36.2	5422.3	85.8	210.9	5.2
B4	35-4-5	335	31.9	5459.1	86.4	228.1	4.8
B6	72-3-14	402	24.6	4798.8	75.9	221.7	4.3
TA 101 (C)	16-3/2	650	26.7	4701.8	74.4	273.8	3.4
B9	K-4	535	23.3	4463.9	70.7	150.2	5.9
B8	92-4-6	390	23.1	4324.9	68.4	293.9	3.0
B5	66-3-9	705	22.8	4279.4	67.8	273.2	3.1

[1] Relative yield to TA 103 (100%), the highest-yielding control clone
[2] YE = yield efficiency (yield/stem section)

Table 3. Total average dry bean yield, yield components, vigour and yield efficiency of intermediate clones in the on-farm testing in six commercial plantations during 2003-08

Clone	Code	Pod weight (g)	Wet bean (%)	Dry bean yield (kg/ha)	Yield index[1] (%)	Stem section (cm^2)	YE[2] (kg/cm^2)
TA 203 (C)	33-8/3	385	30.2	8768.8	100.0	174.7	10.0
I10	K 9	415	28.2	7925.6	90.4	246.0	6.4
I9	K 6	414	25.9	6627.7	75.6	195.8	6.8
I2	17-2-16	455	26.8	6273.5	71.5	200.0	6.3
I6	36-3-2	595	29.4	6082.1	69.4	201.1	6.1
I1	16-3-1	635	25.9	5743.4	65.5	167.3	6.9
I3	21-4-8	375	30.0	5821.6	66.4	211.4	5.5
I4	24-2-8	325	19.2	5297.6	60.4	218.3	4.9
I5	24-4-5	355	34.0	5900.3	67.4	180.1	6.6
I8	92-4-15	411	26.3	5279.4	60.0	182.8	5.8
I7	73-2-3	450	25.7	5256.5	66.8	181.8	5.8

[1] Relative yield to TA 103 (100%), the highest-yielding control clone
[2] YE = yield efficiency (yield/stem section)

Among the small clones (Table 4), the highest-yielding were S9 and S7. Their relative yields in relation to the highest-yielding control were 157% and 111%, respectively. S9 is highly tolerant to CPB while S7 is susceptible. Both clones are resistant to Ppr and VSD and partially self-compatible.

Among the very small clones (Table 5), five clones were identified with higher yields than the highest-yielding control TA 302: VS5, VS4, VS8, VS2 and VS1. Their relative yields in relation to the control TA 302 were 124.8, 123.4, 119.9, 112.8 and 106.4% respectively.

Clones VS5, VS4 and VS2 are tolerant to CPB while VS8 and VS1 are susceptible. For Ppr and VSD all the five high-yielding clones among the very small clones are classified as moderately resistant (MR) to highly resistant (HR). The clone VS5 is self-compatible while VS8, VS4, VS2 and VS1 are partially self-compatible.

Table 4. Total average dry bean yield, yield components, vigour and yield efficiency of small clones in the on-farm testing in six commercial plantations during 2003-08

Clone	Code	Pod weight (g)	Wet bean (%)	Dry bean yield (kg/ha)	Yield index[1] (%)	Stem section (cm^2)	YE[2] (kg/cm^2)
S9	63-3-8	505	33.4	9406.7	157.8	138.8	13.5
S7	34-1-13	470	32.4	6671.7	111.9	130.2	10.2
TA 202 (C)	17-14/4	458	27.8	5960.6	100.0	123.1	9.7
S2	16-4-2	615	28.8	5952.4	99.8	154.5	7.7
TA 305 (C)	73-14/1	325	29.1	5610.2	94.1	133.5	8.4
S1	13-1-13	385	29.1	5486.4	92.0	126.3	8.7
S5	31-3-12	470	25.3	5199.9	87.2	127.5	8.2
S6	33-1-10	320	33.3	4970.1	83.3	128.8	7.7
S4	23-2-13	305	28.5	4550.6	76.3	133.7	6.8
S3	17-4-10	440	22.4	4253.2	71.3	156.8	5.4
S8	35-2-10	385	27.5	3717.0	62.3	142.8	5.2

[1] Relative yield to TA 103 (100%), the highest yielding control clone
[2] YE = yield efficiency (yield/stem section)

Table 5. Total average dry bean yield, yield components, vigour and yield efficiency of very small clones in the on-farm testing in six commercial plantations during 2003-08

Clone	Code	Pod weight (g)	Wet bean (%)	Dry bean yield (kg/ha)	Yield index[1] (%)	Stem section (cm^2)	YE[2] (kg/cm^2)
VS4	13-3-2	405	24.9	7311.0	123.4	126.5	11.6
VS8	37-2-10	655	28.8	7107.4	119.9	141.6	10.0
VS5	15-4-7	490	25.1	7398.6	124.8	127.0	11.7
VS2	13-2-2	325	33.0	6688.4	112.8	123.7	10.8
VS1	11-2-10	510	24.6	6306.9	106.4	143.8	8.8
TA 302 (C)	17-7/4	436	29.6	5924.8	100.0	143.2	8.3
VS7	23-3-3	460	24.5	6177.7	104.4	127.5	9.7
VS6	17-3-6	465	28.7	5751.1	97.1	94.8	12.1
VS9	33-1-13	390	28.6	5241.9	88.5	124.8	8.4
VS3	13-2-13	300	23.0	5001.3	84.4	115.4	8.7
VS10	73-3-8	445	22.0	4215.5	71.1	119.3	7.1

[1] Relative yield to TA 302 (100%), the control clone
[2] YE = yield efficiency (yield/stem section)

The results suggest that clones with higher yields than the control clones can be selected. The very small clones were the highest yielders, which can be partly due to the planting density. Confirmation of CPB tolerance is awaited before commercial release of the best clones can be decided.

General combining ability testing of T x UA clones

- **Materials and methods**

The best 40 Trinitario clones selected in an observation plot were used in crosses with UA tester clones (parents used in the seed gardens for commercial hybrid production). The crossing programme was done in two parts, Part A and Part B:

- Part A was done using 10 Trinitario (males) clones with five UA clones (females), totalling 50 hybrid families. Four commercial SG2 crosses (KEE 12 x KA 2-106, KEE 42 x K 82, KEE 42 x KA 2-106 and KEE 43 x K 82) were used as controls. The hybrids were planted in 5 replications of 16 trees per cross at a density of 833 trees/ha (4 m x 3m spacing).
- In Part B, 32 Trinitario clones were used. All clones were randomly crossed with KEE 12, KEE 42 and KEE 43 to produce 60 experimental hybrids. More clones were crossed with KEE 12 than with KEE 42 or KEE 43. The SG2 crosses KEE 12 x KA 2-106, KEE 42 x K 82, KEE 42 x KA 2-106 and KEE 43 x K 82 were used as controls. Part B was planted in 4 replications with 16 trees/replication at a density of 833 trees/ha (4 m x 3 m spacing). Yield observations started in 2003.

- **Results**

- In Part A, K 72-124 was the best donor for high yields among the Trinitario clones, followed by T 45 and NAB 11. KEE 12 was the donor for high yields among the Upper Amazonian females followed by KEE 42 and KEE 23 (Table 6). The five best combiners for yield among the hybrids in Part A were K 72-124 x KEE 43, K 72-124 x KEE 12, K 72-124 x KEE 42, T 45 x KEE 12 and T 45 x KEE 47.

Table 6. Total average yields (2004-09) of ten selected Trinitario clones tested with five Upper Amazon tester clones (Part A) (values obtained for the best combiners are shaded)

Males	Females					
	KEE 12	KEE 23	KEE 42	KEE 43	KEE 47	Average
TAU 4	5137.7	4282.1	3526.6	3840.2	5207.7	**4398.8**
WOK 30	3025.0	3632.1	3518.0	3760.4	2875.1	**3362.1**
NAB 11	4809.0	4204.4	4646.2	4877.9	4100.2	**4527.5**
RM 8	3515.3	3563.5	4520.3	3807.1	3405.8	**3762.4**
T 11	4364.9	4835.2	3998.2	4313.8	3704.4	**4243.3**
T 45	5700.5	3690.0	4628.3	4458.4	5213.4	**4738.1**
K 72-124	5940.8	3973.7	5784.2	6303.0	4360.9	**5272.5**
L 14	4619.2	4006.5	3250.0	3886.6	4098.5	**3972.1**
B 72	3962.7	4497.4	3926.1	4101.2	4619.1	**4221.3**
RM 1	4014.2	4557.5	4139.0	1276.6	3061.5	**3409.7**
Average	**4508.9**	**4124.2**	**4193.6**	**4062.5**	**4064.6**	
Controls						
KA 2-106	4008.7		3426.9			**3717.8**
K 82			4153.7	4153.0		**4153.3**

- In Part B, a total of 19 hybrids were identified with yields higher than the highest-yielding control (KA 2-106 x KEE 12) (Table 7). Seven of the hybrids out-yielded the highest control by more than 20%. Total yields for the seven hybrids ranged from 5137 kg/ha (KEE 12 x TAU 4) to 6303 kg/ha (KEE 43 x K 72-124).

Table 7. Total yield, annual yield, yield index and stem section of 34 highest-yielding experimental hybrids (2004-09) in Part B

Cross	Pod weight (g)	Wet bean weight (%)	Total yield 2004-09	Annual yield	Yield index[1]	Stem section (cm^2)
KEE 12 x RAM 2	390	31.4	7114	1422.8	133.7	80.5
KEE 43 x T 13	664	30.6	6712	1342.4	126.2	92.0
KEE 12 x PS 2	412	31.6	6644	1328.8	124.9	88.8
KEE 12 x RM 2	398	29.1	6629	1324.8	124.6	80.0
KEE 43 x L 1	644	30.8	6426	1285.2	120.8	93.7
KEE 12 x NAB 12	403	27.9	6332	1266.4	119.0	63.3
KEE 42 x K 72-15	546	26.9	6243	1248.6	117.3	101.5
KEE 12 x NAB 12	384	27.8	6108	1221.6	114.8	63.3
KEE 43 x T 9	608	32.5	6079	1215.8	114.3	103.2
KEE 43 x WOK 34	613	27.5	6044	1208.8	113.6	97.0
KEE 42 x T 20	565	32.0	6008	1201.6	112.9	93.1
KEE 12 x T 27	474	27.1	5976	1195.2	112.3	91.5
KEE 42 x NAB 12	535	28.5	5896	1179.2	110.8	102.6
KEE 42 x WOK 20	520	28.3	5828	1165.6	109.5	99.8
KEE 12 x WOK 1	390	29.6	5799	1159.8	109.0	72.1
KEE 12 x T 13	414	31.1	5783	1156.6	108.7	67.9
KEE 42 x B 18	575	28.8	5458	1091.6	102.6	78.0
KEE 12 x K 72-15	368	28.5	5413	1082.6	101.7	71.2
KEE 43 x B 70	691	26.9	5397	1079.4	101.4	76.5
KEE 43 x K 82 (Control)	**517**	**27.3**	**5320**	**1064.0**	**100.0**	**98.6**
KEE 12 x K 72-49	364	27.6	5312	719	99.8	63.3
KEE 12 x L 49	403	27.9	5148	690	96.8	67.0
KEE 12 x JAH 9	380	31.0	5129	608	96.4	65.1
KEE 42 x B 4	576	27.6	5100	664	95.9	76.0
KEE 42 x K 82	572	29.0	5097	656	95.8	90.4
KEE 42 x JAH 9	579	30.6	5045	670	94.8	97.0
KEE 12 x WOK 20	404	29.6	5038	653	94.7	72.6
KEE 12 x K48 79	452	29.0	4979	577	93.6	80.0
KEE 12 x T 20	382	31.9	4960	631	93.2	71.2
KEE 12 x KA 2-106 (Control)	**375**	**27.2**	**4888**	**630**	**91.9**	**75.0**
KEE 42 x WOK 1	579	31.5	4867	597	91.5	98.6
KEE 43 x RAM 2	661	28.5	4842	586	91.0	100.9
KEE 42 x KA2 106	521	31.7	4835	610	90.9	91.5
KEE 43 x MAI 9	649	30.4	4801	640	90.2	108.4
KEE 12 x T 49	364	27.4	4796	690	90.2	74.1
KEE 42 x KA 2-106 (Control)	**543**	**28.1**	**4748**	**610**	**89.2**	**91.5**

[1] Yield index of selected hybrids relative to the best control (100%)

Impact of the project

The CFC/ICCO/Bioversity project was highly valued for both direct and indirect effects on the breeding programme and on the Institute as a whole. Regarding indirect and desirable impacts, the project:

- Supported major activities of the breeding section and enabled continuity of activities in spite of poor research support from the Government for vital research. Flexibility of the project ensured the maintenance of some of the vital section research activities.
- Provided exposure to technical information available abroad through collaboration and direct communication with project collaborators.
- Allowed the identification in the ENBP on-farm trials of new potential hybrid clones which are higher-yielding than the best control clones.
- Made possible the identification of the new potential hybrids in activity 1.3.2. Not only are the hybrids promising but some clones are also expected to be selected from the best hybrids under the activity.
- Provided an avenue for exchange of ideas through constant communication with the Project Coordinator and during his field visits, and enabled improved communication through the availability of broad band for fast Internet communication.
- Encouraged better team work between other disciplines involved in the project (Pathology and Cocoa Quality sections).
- Provided the opportunity to incorporate CPB testing into breeding activities by initially starting with project activities. Screening technologies were fine-tuned, resulting in the selection of 15 potential clones for CPB tolerance, now under multilocation testing.

Farmer participatory and on-station selection activities carried out at Universidad Nacional Agraria de la Selva, Peru

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Abstract

A final report of activities carried out at Universidad Nacional Agraria de la Selva (UNAS) from 1 June 2004 to 31 May 2009 in the framework of the CFC/ICCO/Bioversity project is presented here. We currently have 90 selections collected from farmers' fields according to participatory selection approach, and 45 trees which were selected from a 14-year-old hybrid plot. Likewise, 28 clones (hybrid selections) and 22 clones (selections from farmers' fields) from the Tulumayo station have been evaluated.

A Regional Variety Trial (RVT) with 21 hybrid progenies (7 introduced and 14 local), showed differences in productivity and its components and good tolerance to witches' broom (WB). On the other hand, some promising clones from the "S" and "C" collections showed superior flavour traits. In addition, a set of seven three-way hybrids which combine moderate resistance to WB and fine quality was generated; these hybrids have been established in the field for evaluation. Sixty hybrid progenies remaining from the RVT and other local progenies were planted in a high density plot and will be evaluated soon.

Results regarding WB infection at fans and cushions level, in the RVT as well as in the clone observation plots, are reported here. Another trial on screening for resistance to WB, at seedling stage from open-pollination selected trees, under high inoculum concentration, showed wide differences between hybrid progenies.

All the researchers of the team were trained, either in Brazil, Ecuador or Costa Rica, and participated in several meetings funded by the CFC/ICCO/Bioversity project.

Introduction

Production constraints

At national level, the productivity of cocoa is highly variable, between 250 and 2500 $kg.ha^{-1}.year^{-1}$. This depends on the variety used, husbandry practices, disease incidence (mainly witches' broom (WB) and moniliasis) and the edapho-climatic conditions of the cocoa-growing areas. The diffusion of CCN 51, a highly productive clone but with regular quality traits, is causing erosion of the native cocoa varieties and of the traditional hybrid varieties known for their superior cocoa quality traits. The efforts to obtain productive varieties with good quality traits remain limited and insufficient to sustain the increase in the cocoa production area observed over the last 5 years. There is no national programme dealing with cocoa. The lack of improved varieties with high yield, good disease resistance and quality traits is a major limiting factor in the efforts to increase cocoa productivity and places Peru as an exporting country of fine-flavour cocoa.

Objectives of selection of new cocoa varieties

The major objectives of the cocoa breeding programme of the Universidad Nacional Agraria de la Selva (UNAS) are to obtain improved varieties that satisfy the cocoa producers, the cocoa industry and the consumers. The programme uses: (i) the wide genetic variation found in hybrid populations growing in extended areas of the country, (ii) the cocoa collection in Tingo Maria, (iii) the advances made in cocoa selection and (iv) international support from various organizations: Bioversity, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France, Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), Brazil, Centro Agronómico Tropical de Investigación y Enseñanza, (CATIE) Costa Rica, Cocoa Research Unit, University of the West Indies (CRU-UWI), Trinidad, United States Department of Agriculture (USDA) and others.

The specific objectives at medium term are to:

- Select superior trees in traditional cocoa varieties, with good levels of tolerance to diseases and fine quality bean traits;
- Create hybrids and select ortets (elite clones) with high yield, resistance or tolerance to diseases and superior organoleptic quality;
- Increase the genetic basis of cocoa in Peru through collection of new native materials and introduction of new materials from abroad or of local origins;
- Support the efforts of public and private institutions to safeguard native landraces; and
- Train extensionists and cocoa producers in the identification and selection of superior trees to be multiplied and conserved as superior clones.

Current status of cocoa varieties

Of the ca. 80 000 ha of land planted with cocoa in Peru, 63% is still made up of the traditional varieties (or 'Criollos') propagated by seed, between 35 and 80 years old; these include landraces (e.g. cultivars 'Porcelana' and 'Chuncho') as well as traditional hybrid varieties produced locally or introduced. The CCN 51 variety occupies 36% and 1% is planted with Trinitario clones (ICS 1, ICS 6, ICS 95, etc.) and Forastero clones (IMC 67, Pound 7, EET 400 and TSH 565).

Unselected varieties

The continuous introduction of cocoa varieties from abroad over more than 50 years has permitted the distribution of traditional varieties and hybridization with local landraces. With the movement of germplasm within the country, this created large opportunities for recombination, resulting in highly variable populations which have not yet been sufficiently characterized and evaluated (Garcia 2008). At least two native landraces ('Porcelana' in the North and 'Chuncho' in the South-East Amazon region) have preserved their original traits and should be further evaluated for their potential use in breeding. Specific measures for the protection of these native landraces are required.

Selection of new varieties

The introduction of varieties started with the arrival of the 'Pound' selections from Iquitos to Tingo Maria at the end of the 1940s. In 1953, the National Project to Improve Cocoa Germplasm introduced, among others, ICS clones (1, 6, 39 and 48), UF clones (2 and 613), Scavina clones (6 and 12) and EET clones (59, 61 and 82). This strategy was continued until the early 1960s, when the first hybrids were created at the Estación Experimental Agrícola (EEA) Tulumayo; they were distributed to the Upper Huallaga and to the valley of the river Apurimac-Ene (Ayacucho). In those regions predominates a mixture of six hybrid varieties: SCA 6 x ICS 1, SCA 6 x ICS 6, SCA 6 x IMC 67, ICS 1 x Pound 7, UF 613 x Pound 7 and UF 613 x Pound 16. These hybrids were obtained by Hurtado (1963) and evaluated during seven years at Tulumayo station (Laos 1991).

In the southern cocoa-growing region of Cusco, hybrid seed from Brazil was introduced but was not distributed because of its high susceptibility to WB. The native landrace of 'Chuncho' cacao is therefore still the predominating cocoa variety.

With the exception of the cocoa-growing area of Piura, where the local landrace 'Porcelana' is cultivated, the variety grown in the majority of the other cocoa-growing areas is the traditional cocoa variety called 'Criollo' or 'Común' cocoa.

Between 1987 and 1989, a United Nations project reintroduced more Trinitario, Forastero and Nacional cocoa clones and collected germplasm from the Huallaga and Ucayali-Urubamba river basins. The material collected was established in two genebanks in Tingo Maria and in Cusco, and in five clonal seed gardens in the Peruvian Amazon for its characterization, evaluation and generation of elite varieties.

In view of the high susceptibility to WB of the seed that was introduced from Brazil in the middle of the 1990s, and which led to the abandoning of recent plantations, it was decided to introduce the CCN 51 variety from Ecuador, because of its high yield and moderate resistance to WB. This variety has increased its area, often by replacing the traditional varieties, and now occupies about 36% of the cocoa-growing area in Peru. According to the cultural system, yields vary between 800 and 2500 kg/ha.

Other varieties have been recommended to be grown together with CCN 51, such as ICS 1, ICS 6, ICS 95, UF 613, TSH 565, IMC 67, Pound 7 and EET 400.

In 1995, after several years of observation of the germplasm collections, a crossing scheme was carried out based on geographical origins and genetic distance between the parental clones. Fifty-four hybrids were created and evaluated in the EEA-Tulumayo.

Multiplication of recommended varieties

The clonal varieties are multiplied by grafting. At first, budding was used but it was substituted by top-grafting or side-grafting. Results with rooted cuttings have been poor, and this technique requires more investment. Somatic embryogenesis is now being experimented; it is hoped that once mastered, this technique can facilitate the quick multiplication of elite trees.

Participatory selection activities with farmers

Participatory selection activities were carried out in 26 farms from different districts of the province Leoncio Prado (Huánuco), of the Tocache y Uchiza (San Martín) districts and of the Irazola (Ucayali) district. As a result, 90 promising trees were identified and collected. These were multiplied by top-grafting and 10 grafted plants per genotype were established in a clonal observation trial in Tulumayo. The results of the evaluation of these 'C' clones are presented below.

On-station selection trials at EEA Tulumayo

- **Evaluation of clones**

During 3 years, 28 selected hybrid trees, 11-13 years old, were characterized and evaluated. Five trees yielded more than 2 kg of dry beans per tree and per year. At the end of the project, 45 selected hybrid trees were established in clone observation plots in Tulumayo. The first year of production (2008-09) of 28 'S' clones showed considerable variation in yield and WB incidence. In the same period 22 'C' clones, selected in farmers' fields, were evaluated for yield and disease incidence. Again, yield and WB incidence varied widely among these clones. It is intended to establish a multilocation clone trial to compare the best clones with CCN 51.

Another aspect evaluated in 2008 was the flavour quality of the cocoa pulp, as possible indicator of the flavour of the cocoa liquor. Among 16 'S' and 'C' clones, 3 were showing the same floral flavour as EET 62, a variety known to produce floral cocoa liquor in Ecuador. The CCN 51 clone showed low scores on fruity and floral flavour traits, and is known to produce regular flavour quality.

Twelve 'S' clones and eight 'C' clones were studied for self-incompatibility. Four 'S' and three 'C' clones were found to be self-compatible.

- **Evaluation of hybrids**

In 2008, the evaluation of the Regional Variety Trial (RVT) started. This trial contains 21 hybrid progenies (7 introduced and 14 of local origin). Yield varied widely among the hybrids, but the differences were not statistically significant due to the high coefficient of variation. Incidence of WB was low, and moniliasis was nearly absent owing to the young age of the trees.

Resistance studies

An early screening procedure for resistance to WB was tested by field planting of seedlings in between adult cocoa trees with high natural WB incidence. The genetic materials used were 71 open-pollinated fruits collected in the RVT and from 'S' clones. The progenies were established in December 2008 using a randomized block design with three replicates of 10 seedlings each planted at 0.4 x 0.4 m distance. Evaluations were carried out until May 2009. The high incidence of infection (60 to 95%) shows that this is a promising method for the selection of resistant seedlings.

Impact of the CFC/ICCO/Bioversity project

At international level, the project:

- permitted us to be part of an international cocoa research effort,
- reinforced the research capacity of the staff involved in the project,
- allowed UNAS to be recognized by the international scientific community,
- permitted the publication of results in newsletters or proceedings of international research conferences, and
- facilitated exchange of cocoa germplasm with other research institutes in the region and also with the quarantine facility at Reading University.

At national level, the following achievements were obtained:

- leadership of UNAS in cocoa breeding research,
- reinforcement of the cocoa genebank of UNAS in Tingo Maria,
- presence of the institute in the Tulumayo research station and re-launching of cocoa research on this station,
- laying down of foundations for the future multilocation evaluation of new cocoa varieties with good yield, resistance to diseases and pests and with good flavour traits,
- generation of technological transfer through training of farmers and extensionists, publication of technical bulletins and cocoa catalogues, and organization of workshops on participatory cocoa selection, and
- establishment of strategic alliances with national research and development institutes (Instituto Nacional de Investigaciones Agropecuarias (INIA); Ministerio de Agricultura-Servicio Nacional de Sanidad Agraria (M.A.-SENASA); Desarrollo Alternativo de la Comisión Nacional para el Desarrollo y Vida sin Drogas – Programa

de Desarrollo Alternativo Participativo (DEVIDA-PDAP); regional and local governments).

Research needs

As research priorities the following activities are proposed:

Genetic resources

- Carry out molecular analyses of the Huallaga, Ucayali, Urubamba, International, Marañón, Amazon and local collections;
- Re-introduce Amazon germplasm and introduce foreign germplasm in order to increase the genetic basis of cocoa in Peru;
- Evaluate and enrich the germplasm collections established by the United Nations Development Programme (UNDP), Instituto de Cultivos Tropicales (ICT) and SENASA; and
- Promote the collection and conservation of native cocoa varieties in Peru.

Breeding

- Reinforce the participatory selection approach;
- Identify new sources of resistance to witches' broom and to moniliasis;
- Implement multilocation clone trials through strategic collaboration with INIA, ICT and other institutions;
- Identify genotypes with superior flavour quality;
- Identify and multiply genotypes that are tolerant to climate change (high temperatures, drought, acidity, etc.);
- Develop and implement marker-assisted selection for yield and disease resistance; and
- Strengthen and implement collaborative agreements with CIRAD, USDA, CATIE and CEPLAC, among others.

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Selection of new varieties on-farm and on-station in Trinidad and Tobago

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Introduction

Trinidad and Tobago is a 100% designated premium producer of fine or flavour cocoa. The average annual production is around 1000 tonnes which is done by approximately 1500 farmers on under 10 000 ha. The cocoa industry is expected to have positive growth within the next 5 years based on significant plantings of young trees within the last 3 years. The Research Division of the Ministry of Agriculture, Land and Marine Resources (MALMR) supports the local industry through the breeding of new cocoa varieties and recommending best agronomy and post-harvest practices.

Cocoa production constraints

Some of the major constraints to cocoa production are:

- Inadequate supply of available agricultural labour
- Low farm productivity
- Ageing farmer population
- High incidence of losses from *Phythopthora* pod rot (Ppr), rodent and bird pests
- Inadequate infrastructure.

Cocoa breeding objectives

These are as follows:

- Improved resistance to Ppr infection
- Maintaining and exceeding a bean size of over 1 g
- Achieving a pod index rating between 12 and 18
- Maintaining fine or flavour status
- Manipulating tree vigour potential to between low and medium.

Current status of cocoa varieties

Unselected varieties

The subject of variety selection in Trinidad and Tobago has remained in the hands of the Ministry and/or that of the breeder for many decades. MALMR has long been known for its very successful breeding programme which started in 1956 and resulted in the production of the Trinidad Selected Hybrids, or TSH varieties. Prior to the TSH varieties, in the 1930s F.J. Pound selected 100 Imperial College Selections (ICS) for good yield potential and apparent field resistance to witches' broom (WB) and these were distributed to farmers with some success. However, the ICSs proved very susceptible to diseases and were low to mediocre in yielding ability.

The TSH varieties are however characterized by the following features:
- Bean size of over 1 g
- Low pod index (10–18)
- Potential yield of 1000–2500 kg/ha
- Early bearing (2–3 years)
- High tolerance to WB
- Good resistance to Ppr (under adequate management)
- Resistance to *Ceratocystis* wilt disease
- Trinitario fine flavour status
- Highly adaptable to local conditions.

Recommended varieties

Through the CFC/ICCO/Bioversity project *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*, the International Clone Trial introduced many varieties which have been evaluated and considered to be valuable germplasm for breeding purposes in Trinidad and Tobago. In the Local Clone Trial, over 21 local clones (TSH varieties) were evaluated and the best performing ones (five in numbers) were selected as new varieties for commercial release to farmers. These are TSH 1344, TSH 1347, TSH 1350, TSH 1352 and TSH 1380. While Intellectual Property Rights are being sought for 11 of these varieties, the remaining 10 varieties are being conserved as valuable germplasm in conservation fields at La Reunion, Trinidad.

Multiplication of recommended varieties

Between the 1960s and the 1990s, eight commercial TSH varieties resulting from the Ministry's breeding programme were available to farmers from the La Reunion Propagation Station of MALMR. These are TSH 730, 919, 1076, 1095, 1102, 1104, 1188, and 1220. Plants can be purchased as grafted clones, seedling trees or clonal cuttings. At the time of writing this report (2010), five new TSH varieties have been planted in seed and clonal gardens at the La Reunion Propagation Station and will soon be released to farmers for planting.

Farmer participatory selection activities

Farm survey

A farm survey was conducted in 2006 on 102 farms in Trinidad. Results were processed and published by INGENIC in a paper titled *Farm practices, knowledge and use of cocoa planting material in Trinidad: A survey report* (Maharaj et al. 2009).

The results showed that 57% of farmers had no knowledge of varieties which they grow while 43% identified either TSH or ICS or Callabacillo types. Eighty-three percent of the farmers surveyed identified the presence of interesting trees on their holdings; within this group of farmers, 84% used the criterion of high-yielding ability and 21% that of black pod disease resistance.

The farm survey formed the basis to launch a major germplasm collection initiative from farmers' fields and the utilization of participatory approaches.

Farm selections

These included 101 farm selections (FSs) from Trinidad and 15 from Tobago; the latter possessed a fair amount of Criollo ancestry based on their light-coloured cotyledons. Eighty-one FS were cloned in a Farmers Budwood Garden at La Reunion and 75 were established in the Farmers Selection Observation Plots (FSOPs).

Thirty FSs were phenotypically characterized and showed a mean bean number per pod of 46, mean cotyledon weight of 1.15 g and pod index of 22.1. Twenty-five FSs were tested for Ppr resistance using the detached pod test (DPT); 28% were moderately to highly resistant, 40% partially resistant and 32% moderately susceptible. Forty FSs were prepared for molecular analysis.

The FSs represents a significant collection made in this project with a tremendous genetic potential for current and future utilization.

On-farm trials

Three on-farm trials (OFTs) have been established at Gran Couva (2007), Biche (2007) and Tamana (2008). On all three OFTs, 20 best farmers' and 10 best breeders' selections were established for evaluation and eventually for farmers to indicate their own preferences. Farm selections were made based on criteria of acceptable DPT ratings, high bean coefficients, prolific bearing and Trinitario ancestry.

Preliminary data collection commenced in 2010, providing the following evidence:

- Some FSs were early bearing with good pod counts, e.g. BMT3, EEGC1, BMT5, EEGC3.
- Breeders' types were much less precocious.
- Precocity of some FSs varied between both locations.
- Vigour of some FSs varied between both locations. Vigorous types were also early-bearing.
- There was a high rate of replacement of trees on all OFT locations (5-15%).

Management of the OFTs is ongoing between the farmers and research teams.

Participatory activities

Two multi-disciplinary stakeholder groups were established by MALMR: the Cocoa Steering Committee and the Technical Working Group. Both have the responsibility to plan and execute field days on the OFTs using Discovery Based Learning methods. Six field days were held since mid-2008 with over 300 farmers trained in good agricultural practices (GAPs).

On-station breeding efforts

Clone selection

Three breeding programmes are ongoing for the selection of clones at La Reunion as follows:

1. Further recurrent selection of best performing progenies from the TSH breeding programme; these progenies are presently being evaluated as T-Types and will eventually be assigned a TSH 1400 code.

2. Evaluation of 10 TSH x TSH crosses for single tree selection. These crosses are:

TSH 919 x TSH 1363	TSH 1102 x OPEN
TSH 1102 x ICS 1	TSH 1220 x OPEN
TSH 1078 x TSH 1220	TSH 1188 x OPEN
TSH 1188 x TSH 919	TSH 1076 x OPEN
TSH 1220 x TSH 1095	TSH 919 x OPEN

3. Joint collaborative project between MALMR and the Cocoa Research Unit, University of the West Indies (CRU-UWI) to enhance the black pod disease resistance of the TSH clones by way of hybridization between TSH clones and best performing progenies from the CFC Germplasm Enhancement Project (GEP/CRU).

Hybrid selection

Two ongoing programmes have resulted in a range of superior hybrid populations, some with improved resistance to the *Phythopthora* disease. These are from TSH x (PA296 x PA171), TSH x (PA137 x PA115), TSH x (PA171 x PA63) and TSH x ICS1 crosses. A selection programme of back-crossing between the PA crosses and TSH is to commence.

Lessons learned

- Preparing, planning, implementing, evaluating and reporting on an international project of this magnitude;
- Networking with various international colleagues and institutions;
- Managing large scientific cocoa trials;
- Understanding new approaches to cocoa breeding systems and management;
- Using new cocoa propagation techniques;
- Using specific experimental procedures and protocols;
- Organizing data management and undertaking statistical analysis of large trials;
- Preparing and presenting scientific reports and papers.

Conclusions and perspectives

Impact of the CFC/ICCO/Bioversity project

- Improvement of cocoa planting materials to farmers.
  Five new improved varieties are being registered for Plant Breeder's Rights and will be available to Trinidad and Tobago farmers in 2011.
- Improvement of the local cocoa breeding programme.
  Through the formalization of the Local Clone Trial, 21 local clones were rigorously assessed for growth, yield, flavour and disease resistance traits.
- Improvement of the infrastructural facilities.
  These concern the Cocoa Research Section, MALMR, i.e. laboratory and field facilities.
- Increased awareness and collaboration among cocoa researchers and stakeholders in the country (CRU, Cocoa and Coffee Industry Board (CCIB) and MALMR).
- Increased access to a wider range of cocoa germplasm for the benefit of breeders.
- Facilitation of the direct involvement of farmers in (a) the choice of variety; (b) participatory training methods such as Discovery Based Learning techniques conducted at Field Days.
- Increased human building capacity among all levels of staff at MALMR.
- Transfer of new technologies such as quality and disease testing methods.
- Increased collaboration between national and international research institutes.

Perspectives

The support for the continuation of ongoing activities can be classified as follows:

- **Financial:** commitment of partial support from the Government of Trinidad and Tobago.
- **Human resources:** staff associated with project will continue to support ongoing activities as the project has now been incorporated into the MALMR's Programme of Work.
- **Collaborative technical support** from research institutes such as CRU, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France), United States Department of Agriculture (USDA) as necessary.

Emanating from the CFC/ICCO/Bioversity project, a new breeding project entitled "Improvement of black pod resistance in the Trinidad Selected Hybrids (TSH)" commenced in 2007 with the CRU-UWI as joint partner. Resistant genotypes identified from the Germplasm Enhancement Project (CRU) were hybridized with the Trinidad Selected Hybrids (MALMR) and extensive screenings of hybrid seedlings by CRU are presently taking place. It is hoped that new TSH types with improved resistance to black pod disease will result.

It is envisaged that further regional collaboration on breeding for resistance to diseases (frosty pod/witches' broom), namely, with the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE, Costa Rica) would continue and be expanded. It is also expected that a new ICCO project on Production and Trade of Sustainable Cocoa in Latin America and Asia would be initiated.

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Selection of new varieties on-farm and on-station in Venezuela

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Introduction

Cocoa is a high-priority crop in Venezuela, one of the few countries with genetic resources of fine quality cocoa. The major local research institutions have been involved throughout their history in research oriented to improve the output of this socio-economic activity. During the last decade, there has been increased commitment in a very active programme to rescue the genetic patrimony of the *Theobroma cacao* species, and particularly to develop methods using this valuable germplasm for the breeding of new varieties to be made available to farmers. To this purpose, national institutions such as the Fondo Nacional de Ciencia, Tecnología e Investigación (FONACIT), Instituto Nacional de Investigaciones Agropecuarias (INIA) and Fundación para el Desarrollo de la Ciencia y la Tecnología del Estado de Aragua (FUNDACITE-Aragua) have been working in collaboration with national and international institutions such as the Common Fund for Commodities (CFC), Bioversity International, the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) and the International Cocoa Organization (ICCO) and research institutions from Asian, African and American countries. One additional reason to justify the country involvement in this project is the need to satisfy the growing demand for new planting materials required to replace old trees, fill empty spaces in old plantations, and establish new plantations. All these activities are supported by the Venezuelian government's investment into cocoa development programmes.

The main breeding objectives were to select for high-yielding clonal varieties with good yield, resistance to pests and diseases and high flavour quality. To achieve this, INIA established two local clone trials, one in Miranda and one in Aragua. A large plot of hybrid cocoa varieties was also set up in Miranda, with the aim of selecting individual outstanding trees from the best hybrid families. On-farm trials were also established to compare clonal breeders' selections with farm selections.

Current status of cocoa varieties

Most of the cocoa grown in Venezuela is made up of unselected varieties of the Trinitario type and of selected hybrids distributed from the 1960s and 1970s onwards. The hybrids consisted of crosses between Upper Amazon and Trinitario parents (IMC 67 and IMC 11 as female parents and OC 61, OC 77 and ICS 6 as male parents). These hybrids were reproduced by manual pollination in seed gardens. Although these hybrids were high-yielding, they were susceptible to the major diseases: *Moniliophthora perniciosa* (witches' broom, WB) and *Phytophthora* spp. (black pod, BP). Another disadvantage of the hybrids was their high vigour, which makes management of the plantations more difficult.

Participatory selection and validation of promising planting materials on-farm

Survey of planting material present on farms and of criteria applied by farmers for choosing new planting materials in their farms

The field survey in INIA-Miranda was completed with 60 farms responding to the questionnaire. The preliminary analysis of the results indicates that clones make up less than 10% of the material planted. Most of the plantation trees originate from seeds and in most cases the farmers do not know their origin. For replanting, farmers use seeds from their own farm, considering precocity, high production, big seeds, big fruits with thin husk, medium-sized trees and few disease spots on fruits. Incidence of WB was observed in 75% of the surveyed farms and incidence of BP in 100%. For both diseases the magnitude of damage varied among farms. The main pests present in the farms were *Steirastoma breve* and fruit borers, also observed with a variable incidence. Regarding new varieties, 85% of the farmers would like to have 'Criollo' materials. As for propagation methods, 85% do not know the benefits of grafted materials, but 100% would like to learn the technique.

At INIA-Centro Nacional de Investigaciones Agropecuarias (CENIAP), the 50 farm visits originally planned were carried out. The information obtained and preliminarily analyses show that almost 100% of the trees were originated from seeds. About 80% of farmers report that the origin of their trees was the extension programme from the Ministry of Agriculture (Cacao Monasterio), i.e. selected hybrid cocoa varieties. The size of 70% of the farms ranges from 1 to 5 ha. The total area for the northern coast of Aragua is near 1000 ha. The average yield is 244 kg/ha. Nearly 70% of the farmers are over 50 years old. In most cases the farmers plant seed from their own farm trees. The criteria used include precocity, high yield, big seeds, big fruits with thin husk, small-sized trees and resistance to fruit diseases and pests. As for pests, the main problems are pod borer and BP disease. As for type of planting material, 20% would prefer seeds or plants from the Ministry of Agriculture programme, 32% are willing to use clones and 28% could use both seed plants or clones. Nearly 44% are interested in new varieties. The criteria applied to select the materials to be included in the on-farm trials were: high fruit production (>30 fruits per tree), fruits with abundant and big seeds, and finally light-coloured and aromatic seeds.

Identification and collecting of promising mother plants in farmers' populations, according to selection criteria applied by farmers and researchers

At INIA-CENIAP from 70 trees recently identified in farmers' fields, 45 were planted in on-farm participatory selection trials. For the rest of these materials, no further action other than periodical observations was taken. At INIA-Miranda, 48 of the identified trees were used to plant on-farm participatory selection trials, 10 trees were collected and multiplied on the station nursery. Other identified trees are kept under observation at the original sites, before any decision is taken to collect and multiply them in on-station observation plots.

Establishment of multilocation on-farm variety trials with eight replicates each

Five replications have been established and managed at INIA-Miranda with 48 on-farm selected clones plus 10 INIA selections at the following localities: Tacariguita, El Tesoro, Las Toros, Mango de Ocoita and Santa Barbara. Five replications have been established by INIA-CENIAP at the following localities: Aponte-La Isleta, La Esmeralda, Hacienda Cata, Cumboto-El Paraiso and Choroni, with 45 on-farm selected clones plus 10 INIA selections.

All these trials were established together with the farmers committed to the activity. They offered to take care of the trials and record observations together with researchers.

Maintenance and observations to be carried out in on-farm variety trial plots: growth, yield, diseases and pests incidence

At INIA-CENIAP, four of the five replications planted in the selected farm/locations are doing well; the fifth one (Aponte-La Isleta locality) suffered from a lack of interest from the farmer. It has to be maintained by the researcher and technicians from INIA-CENIAP. At two localities (La Esmeralda and Hacienda Cata) the precocious materials have borne fruits by 2009. At Cumboto-El Paraiso the trees bloomed but did not bear fruit, and at Choroni only a few local materials had started to bear fruits.

At INIA-Miranda, diameter of stem and height of plants were recorded at the five localities or replications Tacariguita, El Tesoro, Las Toros, Mango De Ocoita and Santa Barbara. Differences were observed among materials and localities. The materials offered by INIA had higher values for diameter and plant height, and the highest values were observed at the localities El Tesoro and Tacariguita. The same situation occurs for fruit bearing.

Nursery multiplication, field planting and maintenance of interesting planting materials collected in farmers' fields in observation plots on-station

At INIA-CENIAP the observation plots of materials transferred from Ocumare de la Costa into the INIA-CENIAP experimental field are doing well. The following materials show early and acceptable production of fruits: CHO 42, OC 67, CHU 120, 0C 60, LS 2, CNM 4 and VID 1. Among these, clones OC 67, CHU 120 and OC 60 show equally good behaviour in the Local Clones Trial at Ocumare de la Costa Experimental Field.

At INIA-Miranda, the observation plots of clones derived from trees selected on-farm by farmers and researchers recently and in former occasion are under observation. Data have been collected in 2008 for yield and associated traits on 120 clones. Yield varied from 25.9 to 1261.2 kg/ha. Nine clones with the best yield and acceptable bean and pod indices were selected.

Preliminary evaluation of 100 clones derived from trees selected on farmers' fields, by means of field disease incidence and early resistance tests

At INIA-CENIAP the planting materials selected for the participatory trials on farmers' fields are being observed for disease incidence, particularly BP and *Lasiodiplodia*.

At INIA-Miranda the materials which have been selected in a participatory way, planted in the on-farm trials plus others previously selected, are being observed for disease incidence and vigour indices in the farm trials and Local Clone Observation Plots (LCOPs) on-station. Regarding incidence of diseases for 2008, 48 clones had no incidence of WB on fruits while for the rest of the clones 2 to 20% of the fruits were affected. For BP 12 clones did not show any incidence while for the rest of clones from 3 to 50% of fruits were affected.

Study of genetic diversity of accessions collected in farmers' fields using SSR markers

For the Local Clone Observation Plot (LCOP) established at INIA-Miranda, 129 clones collected from selected trees in farmers' fields plus 11 reference clones from the germplasm collection were characterized with 23 microsatellite markers (simple sequence repeat, SSR), to quantify their genetic diversity and their genetic similarity. Eighty-one alleles were observed, including 12 rare alleles and 7 low frequency alleles. Nine groups of clones were observed according to their similarities. The clones were identified as hybrids between Criollo cocoa and Forastero cocoa, with varying proportion of either Criollo or Forastero.

Organization of "National Stakeholders" planning workshops

The aim of the workshops was to take decisions on procedures for participatory selection of new varieties and on-farm selection trials, as well as to identify which stakeholders would participate and how many participants were expected. A National Stakeholders' meeting was held at INIA-Miranda on 24 November 2005 and a second meeting was held at INIA-CENIAP in Ocumare de la Costa on 8 December 2005. The activities were completed in both research units involved in the project during the second year.

At INIA-Miranda the Stakeholders meeting was attended by 10 farmers, 5 chocolate producers (small scale), 4 representatives of credit organizations, 4 extension workers and 6 researchers. The presentations dealt with the situation and perspective of the cocoa business, general view of the project and details on progress of the project activities in Miranda. The need for the teamwork between farmers, researchers and extension officers was stressed in all the presentations. During the discussions, the matters of interest addressed by the participants were: need for more localities to be involved, relevance of post-harvest treatment, favourable comments on grafting technique for propagation, and interest in the system applied by INIA for distribution of planting materials to the farmers. The need to define certain rules for participation, which at the end granted accomplishment of the goals through the commitment of all actors involved, was also pointed out.

A similar approach was used for the meeting held by INIA-CENIAP in Aragua. This meeting was attended by 22 farmers, 4 extension workers, 3 researchers and the Mayor of Ocumare de la Costa. The meeting started with a welcome from the head of the farmers' organization, followed by presentations by the National Technical Coordinator (overview of the project) and from the local leader of the project (progress report of the project activities). During the discussions many questions were raised about the advantages or disadvantages of clones vs. plants originating from seed, particularly related to the management of clones to prevent the rootstock to prevail over the graft. Another issue of interest was post-harvest treatment and prices of final product. A visit to the Local Clone Trial experimental field allowed many of the questions about grafting and management of grafted populations to be answered on site. Farmers' comments on the project were favourable. There was expression of interest in covering more sites with the trial.

On-station breeding efforts

Evaluation of the International Clone Trial

The International Clone Trial (ICT) was established in the Padron Experimental Field, in the State of Miranda, by INIA-Miranda, between June 2001 and October 2003, with a field layout of a completely randomized blocks design, 4 replications and 8 trees per plot. This trial included 25 introduced clones and 4 local clones which served as controls. The first significant yield data were recorded during the year 2005. By the years 2007 and 2008 the plantation looked uniform. The analysis of the yield data from the harvests of years 2006 through 2008 shows a consistent group of six clones included within the first six rank positions, namely: MAN 15-2, PA 107, EET 59, Mocorongo, IMC 47 and PA 150, which also appear in that order in the results of the analysis of variance for the combined 4-year data. The average yield for the trial varied from 458 kg/ha in 2005 to 724 kg/ha in 2006, 594 kg/ha in 2007 and 1082 kg/ha in 2008. This indicates a trend towards increased yield with a fall in 2007. Regarding yield components, the first to sixth positions for number of fruits were MAN 15-2, PA 107, PA 150, EET 59, Mocorongo and IMC 47; this result confirms the close relationship between yield and number of fruits. The most outstanding clones for the weight of seeds per fruit were EET 59 and MAN 15-2; for the number of beans per fruit, Mocorongo, MAN 15-2 and IMC 47; for bean index, EET 59 and for pod index EET 59 and MAN 15-2.

The incidence of BP and WB (pod attack) in the State of Miranda varied through the years 2005 to 2008. For BP the highest values were observed in 2005 and 2008, while for WB they were observed in 2008 only. Perhaps the effects of the environmental conditions, particularly rainfall, were the main cause of that variation. The selected clones which scored best for resistance to both diseases on the basis of field incidence on fruits, considering the mean percentage value for the 4 years and the lowest and highest percentage values were: Mocorongo, MAN 15-2, EET 59, IMC 47, SC 10 and OC 61. The clones PA 107 and PA 150 had mean values for BP above 20%; all other clones except EET 59 had mean values for WB below 10%. The field incidence of WB as vegetative brooms (VB) and flower cushion brooms (FCB) show some differences in clones between the two symptoms. The best clones for both types of brooms were IMC 47, MXC 67, WA 40, SCA 6, PA 150, Playa Alta 2 and LCTEEN 46, with total values below 10 brooms. The clones with the highest values for both types of brooms were VENC 4-4, BE 10 and APA 4. The Pearson correlation between vegetative and flower cushion brooms (r=0.51) was significant at 1% probability.

Inoculation tests were performed with zoospores of *Phytophthora palmivora* using two methods: leaf disc method (LDM) and detached pod method (DPM).

For the leaf disc test a 6-point evaluation scale was used (0 to 5). The results were observed five days after inoculation. Three groups of clones were identified:

- moderately resistant (MR)	IMC 47, MAN 15-2, PA 120, SCA 6
- moderately susceptible (MS)	PA 150, GU 255, VENC 22, WA 40, Mocorongo, SC 10, AMAZ 15-15, VENC 4-4, CATIE 1000, LAF 1, EET 59, APA 4
- susceptible (S)	SPEC 54-1, Playa Alta 2, EQX 3360-3, PA 107, LCTEEN 46, BE 10, ICS 43, OC 61, MXC 67.

For the detached pod test, using an 8-point scale (1-8), the clones were grouped as follows:

- moderately resistant (MR)	SCA 6, PA 150, GU 255, WA 40, Mocorongo, MAN 15-2, VENC 4-4
- moderately susceptible (MS)	VENC 22, SC 10, AMAZ 15-15, PA 120, PA 107, CATIE 1000, EET 59, IMC 47, SPEC54-1, EQX 3360-3
- susceptible (S)	MXC 67, Playa Alta 2, LCTEEN 46, LAF 1, BE 10, ICS 43, APA 4, OC 61

The two methods differ in the number of MR clones: for the leaf disc method this category comprised only four clones, while for the detached pod method it comprised seven clones. Both methods coincide in including SCA 6 and MAN 15-2.

Regarding the six clones selected as the best yielders, MAN 15-2 was MR by both methods while PA 150 and Mocorongo were MR by the DPM.

From the 4-year results, we can conclude that 6 of the 21 clones introduced from Montpellier and Reading quarantines had an average yield for the 4 years evaluated above 1000 kg/ha of dry cocoa. They were: MAN 15-2, PA 107, EET 59, Mocorongo, IMC 47 and PA 150. Two of them had bean index values above 3 (PA 150 and EET 59). All of them had pod index values between 22 and 30, with EET 59 and MAN 15-2 having the lowest average values within that range. Four of those clones had an average value of fruits affected by BP below 20% (Mocorongo, MAN 15-2, EET 59 and IMC 47). All but EET 59 had average values of fruits affected by WB below 10%. The clone MAN 15-2 was MR to BP by field incidence, leaf disc and detached pod inoculation. Mocorongo was MR to BP by field incidence and detached pod inoculation.

Evaluation of the Local Clone Trial

The Local Clone Trial (LCT) was established in the Padron Experimental Field, in the State of Miranda, by INIA-Miranda, between June 2001 and October 2003, with a field layout of a completely randomized blocks design, 4 replications and 8 trees per plot, with a planting distance of 2.5 m by 2.5 m and population density of 1600 trees per hectare. This trial included 25 local clones. A similar trial with 22 local clones was established at INIA-CENIAP, following the above layout and characteristics.

For the LCT at INIA-Miranda, the data for the years 2005 through 2008 harvest have been analysed. The yield shows an increasing tendency from 2005 through 2008, from 747 to 1194 kg of dry cocoa. From 2006 through 2008 the following ten clones had yields above 1000 kg/ha of dry cocoa:

PV 1 x IMC 11	O 14 x M 2
ICS 6	SCA 6 x PV 1
IMC 67 x SC10	EET 250
IMC11	OC 61 x PLA 159
EET 332	IMC 67

Two of these clones (PV 1 x IMC 11 and ICS 6) had an average yield above 2000 kg/ha of dry cocoa during 2007 and 2008. These ten clones continue under observation and will be multiplied for further testing on farms, with the participation of farmers, as soon as possible. Regarding the incidence of BP on fruits, the mean of the 4 years for the ten selected clones was below 25% of fruits affected, with the exception of clones IMC 67 x SC 10 and EET 250. In the case of WB, nine of the ten best clones had an incidence on fruits below 10%, ICS 6 being the exception with a mean of 13%, which is still low. Regarding vegetative brooms (VB) and flower cushion brooms (FCB), both had low values during the year 2008, ranging from 0.71 to 1.48 for VB and from 0.71 to 1.61 for FCB. A second evaluation done in May 2009 gave a variation from 0.3 to 17.0 VB per clone and from 0.8 to 61.5 for FCB. From the ten yield-selected clones, all but ICS 6 had less than 10 VB, five had less than 10 FCB and another four had less than 25 FCB. The clone ICS 6 had the highest value of 40 FCB. The Pearson correlation, estimated in 2008, between vegetative brooms and flower cushion brooms (r=0.81) was significant at 1% probability.

At INIA-CENIAP, the first fruits were harvested in 2003; in 2004 19 clones were harvested, with a great variation in the number of trees harvested per clone. The clones CHU 120 and OC 67 were among the most precocious clones. On the basis of the year-to-year and cumulative yields, the clones with the best performance were OC 60, OC 67, SCA 6 x PV 1 and OC 61 x PLA 159. They were always above the mean of the two control clones (OC 61 and SC 10) and the mean of the trial. Regarding the reaction to inoculation of detached pods with *Phytophthora palmivora*, the six best clones in the trial behaved as follows: (SCA 6 x PV 1) as highly resistant and CHO 174, ICS 6, EEM 001 and OC 61 x PLA 159 as resistant.

Hybrid families trial

This trial was established at INIA-Miranda with 60 hybrid families. According to the origin of the parental clones, the families could be classified in three groups: 17 crosses between Trinitario and Amazon clones, 15 crosses between Trinitario clones and 28 crosses between Trinitario, Amazon and Criollo clones. The commercial crosses IMC67 x OC61 and IMC67 x SC10 were used as controls. The families were arranged in a layout corresponding to a completely randomized design, with 3 to 10 replicates per family and 6 trees per plot. The trial was established in a 2.5 ha-field, between October 2000 and July 2001. Observations on early vigour (stem diameter and plant height) as well as diseases and pest incidence (WB, "gota" (cocoa beetle) and stem borers) were recorded during the establishment phase of the trial.

The criteria for selection of the families were yield in kg/ha (Y), bean index (BI), pod index (PI), percentage of BP fruits (BP%F), percentage of fruits affected by WB (WB%F), number of vegetative brooms per plant (VB/PL), and number of cushion brooms per plant (CB/PL).

Population 1 contains 17 families derived from crosses between Trinitario and Amazon cocoa clones. The range of variation for the selection criteria was as follows: Y (537–899), BI (1.3–1.8), PI (17-29), BP%F (4–26), WB%F (9–29), VB/PL (0.08–2.90) and CB/PL (0.15-10.08). From this population there were 4 crosses with an average yield per family between the two control crosses (IMC 67 x OC 61 and IMC 67 x SC 10). They were: Cumbo 177 x (IMC 67 x SC 10), OC 61 x (OC 77 x EET 400), EEM 003 x IMC 67 and EEM 003 x EET 250. Those families also had good values for BI (>1.3) and PI (<22), low incidence of BP and acceptable incidence of WB-infected fruits below 25%. The cross Cumbo 177 x (IMC 67 x SC 10) had a plant highly resistant to BP and from 19 plants evaluated, 58% were within the range of resistance. Also worth to be mentioned were the crosses EEM 003 x IMC 67 and EEM 003 x EET 250 for their good productivity indices and low incidence of BP and WB on fruits and well as vegetative or flower cushion brooms.

Population 2 contains 15 families derived from crosses between Trinitario clones. Five families were among the best ones according to the results from the combined analysis of the two years 2007 and 2008. They were: EEM 003 x OC 61, Cumbo 177 x (Playa Alta 1 x SC 10), OC 61 x ICS 6, EEM 001 x ICS 6 and ICS 6 x EEM 003. These clones also have good bean indices (1.4 to 1.8) and pod indices (19 to 23). The incidence of BP on fruits varied from 3 to 14% and that of WB from 7 to 14%. The incidence of vegetative brooms per plant varied from 0.16 to 3.38 and for cushion brooms from 0.31 to 28.25. The cross OC 61 x ICS 6 was the worst for the latter trait. The other four families had in general good indices for productivity and disease incidence.

Population 3 contains 28 families derived from crosses between Trinitario, Amazon and Criollo cocoa clones. From the result of the combined analysis of years 2007 and 2008, the six families below were selected for their yield performance, as compared with the two control crosses:

(PV 1 x IMC 11) x ICS 6	Cumbo 177 x (PV 1 x IMC 11)
SJU 3 x ICS 6	La Concepcion 164 x (PV 1 x IMC 11)
SJU 3 x OC 61	(PV 1 x IMC 11) x SJU 3

These crosses, besides yield, have good indices on traits associated to yield: BI (1.3 to 1.6) and PI (17 to 22). Regarding BP, the incidence on fruits varied from 5 to 20% and for WB from 9 to 16%. The incidence of vegetative brooms was between 0.19 and 2.90 brooms per plant while for flower cushion brooms it was between 2.03 and 14.03 brooms per plant. The more common female parents for the 15 selected crosses were: EEM 003 (3), OC 61 (2), SJU 3 (2), PV 1 x IMC 11 (2) and Cumbo 177 (2).

On the basis of results from the harvests from year 2006 to 2008, 12 families had been pre-selected from the 60 crosses of this activity, considered as a single population. These families were originated from the crossing of 13 parental clones. The families and their parental clones were:

(OC 61 x PA 159) x (SCA 6 x SC 10)
Cumbo 177 x (IMC 67 x SC 10)
SJU 7 x Parinari 121
IMC 67 x SC 10
EEM 003 x OC 61
OC 61 x EEM 001
SJU 4 x OC 61
Cumbo 177 x (PV 1 x IMC 11)
IMC 67 x OC 61
La Concepcion 164 x (IMC 67 x SC 10)
SJU 3 x (PV 1 x IMC 11)
(PV 1 x IMC 11) x SJU 3
OC 61 x (OC 77 x EET 400)
EEM 001 x Playa Alta 1

Not all the members of this group were eventually selected. It was decided that the families and the parental clones were to be evaluated further for their reaction to BP and studied in detail for incidence of WB, as vegetative and flower cushion brooms. Only 10 families and 11 parental clones were evaluated for resistance to BP. The parents SJU 3, SJU 4 and SJU 7 were not evaluated, because they are not present in INIA-Miranda nor INIA-CENIAP collections. They are kept in San Juan de Lagunillas, an experimental field of INIA-Merida. Twenty-nine individual plants from seven of the evaluated crosses were resistant to BP. Three parental clones (Parinari 121, EEM 001 and Cumbo 177) were resistant to BP. The other eight were susceptible. In the case of BP we are still lacking good sources of resistance. For incidence of WB, 13 hybrid families were evaluated for incidence of vegetative and flower cushion brooms. Three families – EEM 001 x Playa Alta 1, OC 61 x EEM 001 and La Concepcion 164 x (IMC 67 x SC 10) – had less than 1 vegetative broom per plant, three had between 1.4 and 2.9 and six had more than 4.4 vegetative brooms per plant. Two families – OC 61 x EEM 001 and EEM 001 x Playa Alta 1 – had less than 1 cushion broom per plant, four had between 1.9 and 3.7 and seven had more than 10 cushion brooms per plant. These 3 crosses have to be considered in further selection of plants with resistance to WB. The Pearson coefficient of correlation between vegetative and cushion broom incidence was 0.90.

The evaluation of the 60 hybrid families was also carried out. It shows 83% families with less than 1 vegetative broom/plant and 24% families with less than 1 cushion broom/plant. The nine hybrid families listed below had the smallest number of both types of brooms per plant. These nine crosses should also be considered for individual plant selection for resistance to WB.

EEM 001 x EET 250
Playa Alta 3 x (Playa Alta 1 x SC 10)
Cumbo 177 x (OC 77 x EET 400)
La Concepcion 164 x (Playa Alta 1 x SC 10)
EEM 003 x (PV 1 x SCA 6)
Playa Alta 1 x (IMC 67 x SC 10)
EEM 001 x Playa Alta 1
EEM 001 x (OC 77 x EET 400)
ICS 6 x EEM 001.

Evaluation of varieties in the Regional Variety Trial (RVT)

The behaviour of the families regarding incidence of WB and BP, as well as production traits, is still under observation in the trial established at INIA-Miranda. During 2008 all of the 13 families were producing fruits. However the results were too preliminary to consider analysing them. The estimated yield varied from 203.8 to 476.6 kg dry cocoa per hectare, the BI varied from 1.1 to 1.5 and the PI varied from 14.5 to 27.2. Regarding WB, the incidence was low, varying from 1 to 14% of trees with vegetative brooms. Twelve families had less than 10% of fruits affected. For BP, the incidence varied from 3 to 24% of fruits affected.

Seven families had between 3 and 10% of fruits affected. These families will be further observed and tested for resistance to BP.

Conclusions

At the end of the CFC/ICCO/Bioversity project *"Cocoa Productivity and Quality Improvement: a Participatory Approach"*, the INIA-Venezuela research team has several significant results to report.

- Six ICT clones and 10 LCT clones at INIA-Miranda, 4 LCT clones at INIA-CENIAP and 15 hybrid varieties at INIA-Miranda have been selected for further evaluation and/or use in breeding.
- Ten on-farm trials with clones from the research units and clones derived from trees selected by farmers and researchers were established.
- At INIA-Zulia resistance (R) to moniliasis was identified in Criollo Guasare clones SJU 1, SJ 3 and rated MR to MS in SJU 5, SJU 1 and SJU 16. Other Andina Criollo clones rated MR to MS were BOC 05, BOC 10 and 7 BEN.
- The project has also introduced new technologies to farmers in the States of Miranda and Aragua, such as vegetative propagation by grafting and use of clones.
- An MSc thesis at INIA-Miranda has shown resistance to penetration of WB to depend on a major gene, and the extension of disease to be also governed by a major gene.

It will only take further statistical analysis to quantify the repeatability of behaviour of the selected clones and hybrid families. It will also be necessary to use the data available to select the most promising individual plants in the hybrid populations.

EVALUATION OF THE INTERNATIONAL CLONE TRIAL

Comparisons of agronomic and resistance traits for the International Clone Trial

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Abstract

The stability of agronomic and resistance traits for the International Clone Trial over eight countries was compared through linear regression analyses. The number of clones compared varied over the sites from 8 to 18. For average yield, the majority of the coefficients of correlation were positive and significant, showing reasonable stability of this trait over sites. For plant vigour measured by stem girth, the coefficients of correlation were generally positive but rarely significant, indicating interaction between sites and clones. Incidence of witches' broom (WB) disease was compared for four sites (Brazil, Ecuador, Trinidad and Venezuela). Correlations for the total number of brooms, for the number of vegetative brooms and for the number of cushion brooms per tree were positive but not always significant, suggesting some interaction between clones and sites for resistance to WB. In Ecuador, the total number of brooms per tree was significantly correlated with the average weight of the brooms. The numbers of cushion brooms and vegetative brooms were significantly correlated for three out of four sites. For vascular streak dieback (VSD) severity, the coefficient of correlation between two sites in Papua New Guinea was significant, but the correlations between these sites and Malaysia were not significant. The consequences of these findings for breeding are discussed.

Introduction

The main objective of the International Clone Trial (ICT) was to evaluate stability of economically important traits, such as yield, vigour, resistance to diseases and quality. In the current paper, the stability of agronomic and disease resistance traits are compared over eight different sites where the ICT was established.

Materials and methods

The ICT was established at eight different sites: Brazil, Ecuador, Trinidad, Venezuela, Côte d'Ivoire, Ghana, Malaysia and Papua New Guinea. Yield was expressed as dry cocoa yield per tree or per hectare evaluated over 3 to 5 years. Vigour was expressed as stem girth measured 10 cm above the grafting point at the end of the experiment (2008 or 2009). Resistance to witches' broom (WB), caused by *Moniliophthora perniciosa*, was expressed as average number of vegetative and cushion brooms per tree evaluated over 2 to 4 years. In Ecuador the average dry weight of the brooms was also evaluated. Resistance to vascular streak dieback (VSD) disease, caused by *Oncobasidium theobromae*, was expressed by a score ranging from 0 to 5 for disease severity observed over 2 years.

Results were compared by calculating coefficients of linear correlation for those clones that were in common between the sites. The number of common clones varied from 5 to 18, depending on the sites. No coefficient of correlation was calculated for comparisons between sites with less than eight clones in common.

Results

For dry cocoa bean yield, the correlations between sites were nearly always positive and often significant (Table 1). Among the most productive clones were Mocorongo, T 85/799, UF 676, MAN 15-2, PA 107 and EET 59. Among the least productive clones were SCA 6, AMAZ 15-15, APA 4, BE 10, EQX 3360-3, GU 255V, LCTEEN 46, MXC 67, VENC 4-4 and LAF 1. Clones with intermediate yielding capacity were ICS 1, CATIE1000, IMC 47, PA 120, Playa Alta 2 and SPEC 54-1.

Table 1. Coefficients of correlation for yield for the International Clone Trial among seven sites

	Papua New Guinea	Trinidad	Venezuela	Ecuador	Ghana	Côte d'Ivoire
Malaysia	0.50 ns	-	0.57 ns	0.50 *	-0.72 ns	-
Papua New Guinea		0.77 **	0.77 **	0.70 **	0.18 ns	0.52 ns
Trinidad			0.89 **	0.69 **	0.86 **	0.45 ns
Venezuela				0.74 **	0.64 *	0.60 *
Ecuador					0.83 **	0.28 ns
Ghana						0.01 ns

* = significant at 5%
** = significant at 1%
ns = not significant

For vigour, measured as stem girth, the coefficients of correlation were generally positive but low, and significant only for 4 out of 28 comparisons (Table 2). The most vigorous clones were APA 4, VENC 4-4, Playa Alta 2, IMC 47, MAN 15-2, PA 120, ICS 1, EET 59, CATIE 1000 and the least vigorous were AMAZ 15-15, LAF 1 and BE 10. Clones with medium or variable levels of vigour were MXC 67, SPEC 54-1, PA 107, Mocorongo, SCA 6, T 85/799, UF 676, EQX 3360-3, GU 255V, P 7 and LCTEEN 46.

Table 2. Coefficients of correlation for plant vigour (girth) for the International Clone Trial among eight sites

	Malaysia	Ecuador	Venezuela	Trinidad	Brazil	Ghana	Côte d'Ivoire
Papua New Guinea	0.90 **	0.22 ns	-0.15 ns	0.54 *	-0.05 ns	0.01 ns	0.15 ns
Malaysia		0.49 ns	0.63 ns	0.80 *	-0.30 ns	0.41 ns	-
Ecuador			0.19 ns	0.31 ns	0.06 ns	0.23 ns	0.34 ns
Venezuela				0.61 *	0.50 ns	0.23 ns	0.30 ns
Trinidad					0.65 *	0.55 ns	0.47 ns
Brazil						0.49 ns	0.51ns
Ghana							0.69 *

* significant at 5%
** significant at 1%
ns = not significant

For WB incidence, the correlations for the total number of brooms were significant among Trinidad, Venezuela and Ecuador (Table 3). The correlations between these countries and Brazil were positive but not significant. For cushion brooms and vegetative brooms (Tables 4 and 5), the correlations were lower but still often significant among the four countries. There was also a significant correlation between cushion and vegetative brooms for Brazil, Ecuador and Trinidad (Table 6). In average, the most susceptible clones were EET 59, LAF 1, MAN 15-2, UF 676, VENC 4-4 and ICS 43. The least susceptible clones were AMAZ 15-15, GU 255, IMC 47, LCTEEN 46, PA 107, Playa Alta 2 and SCA 6. The SCA 6 clone was highly resistant in Brazil but moderately susceptible at the other sites.

For Ecuador, the weight of dry brooms was also recorded. This parameter appeared significantly correlated with the total number of brooms in Ecuador as well as in Trinidad.

For VSD disease severity, the correlation among the two sites in Papua New Guinea was positive and significant. However, the correlations between Papua New Guinea and Malaysia were not significant (Table 7). The most resistant clones in Papua New Guinea were AMAZ 15-15, MAN 15-2, Mocorongo, SCA 6, T 85/799 and IMC 47. The most susceptible clones were BE 10, CATIE 1000, ICS 1, SPEC 54-1, VENC 4-4 and MXC 67.

Table 3. Coefficients of correlation for the total number of brooms per tree of *Moniliophthora perniciosa* at four sites and for average dry broom weight in Ecuador

	Trinidad	Brazil	Venezuela	Ecuador (weight of brooms)
Ecuador	0.64 **	0.54 ns	0.77 **	0.79 **
Trinidad		0.52 ns	0.57 *	0.59 *
Brazil			0.08 ns	0.56 ns
Venezuela				0.31 ns

* significant at 5%
** significant at 1%
ns = not significant

Table 4. Coefficients of correlation for number of cushion brooms per tree of *Moniliophthora perniciosa* at four sites

	Trinidad	Brazil	Venezuela
Ecuador	0.50 *	0.54 *	0.64 **
Trinidad		0.44 ns	0.62 **
Brazil			0.11 ns

* significant at 5%
** significant at 1%
ns = not significant

Table 5. Coefficients of correlation for number of vegetative brooms per tree of *Moniliophthora perniciosa* at four sites

	Trinidad	Brazil	Venezuela
Ecuador	0.50 *	0.52 ns	0.60 *
Trinidad		0.57 *	0.62 **
Brazil			0.32 ns

* significant at 5%
** significant at 1%
ns = not significant

Table 6. Coefficients of correlation between number of fan brooms and number of cushion brooms per tree of *Moniliophthora perniciosa* at four sites

Ecuador	Trinidad	Brazil	Venezuela
0.70 **	0.77 **	0.77 **	0.37 ns

** significant at 1%
ns = not significant

Table 7. Coefficients of correlation between VSD disease severity between three sites

	Papua New Guinea (Madang)	Malaysia
Papua New Guinea (Tavilo)	0.79 **	0.40 ns
Papua New Guinea (Madang)		0.43 ns

** significant at 1%
ns = not significant

Discussion and conclusions

Dry cocoa yield appeared to be a quite stable trait over the eight sites where the ICT has been established. This would mean that selection for yield at one site may also be useful for other sites. These results also appear to be in agreement with the observation that the high-yielding character of clones such as CCN 51 and PBC 23 is stable over many different environments where these clones have been planted commercially.

Plant vigour, measured as stem girth, was less well correlated over the sites. However, it was possible to identify clones with high vigour and clones with low vigour over most sites. Another group of clones showed to be variable in vigour. The indication that vigour appears to be less stable than yield over sites is possibly related to the fact that vigour is less easily measured for clonally propagated materials, and also to a possible interaction between rootstocks and scions.

For WB, the low coefficients of correlation indicate some interaction between clones and sites. One of the highest interactions was for the SCA 6 clone, still rated as resistant in the trial in Brazil whereas this clone showed moderate susceptibility at the other three sites. The weight of the brooms in Ecuador was positively correlated with disease incidence. This is of interest, as in Trinidad the broom base diameter, which is an indicator of the broom size, is also significantly correlated with field incidence of WB (Surudjeo Maharaj et al. 2009).

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Reference

Surudjeo Maharaj S, Umaharan P, Eskes AB, Thévenin JM, Butler DR. 2009. Inheritance of components of resistance of cocoa to vegetative infection of *Moniliophthora perniciosa* evaluated through agar-droplet inoculations and correlation with field resistance. In: Eskes AB, Efron Y, End MJ, Bekele F. Proceedings of the International Workshop on Cocoa Breeding for Farmers' Needs, 15-17 October 2006, San José, Costa Rica. INGENIC and CATIE, UK and Costa Rica. pp. 142-155.

Comparative organoleptic evaluations of cocoa (*Theobroma cacao* L.) accessions from the International Clone Trial by three sensory panels over two years

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Abstract

The flavour attributes of cocoa liquors prepared from some cocoa clones planted in the International Clonal Trials of eight different countries were evaluated over two years on seven main flavour traits to examine individual and combined clonal and environmental effects. Fermented and dried cocoa bean samples were prepared in each country according to standardized protocols and the samples were evaluated for any defects before liquors were prepared according to a standard method. Sensory evaluation was undertaken by three panels, one consisting of two panellists and two consisting of one panellist. The results showed low coefficients of correlation between panels for the descriptors evaluated. However, the Multiple Factorial Analysis showed some common features between panels, depending on the axes. With regard to specific flavour attributes, acidity, bitterness and astringency were opposed to cocoa flavour intensity whilst floral flavour appeared to be the most independent flavour trait, related mainly to the clone for the two years' data that were analysed. Significant year and country effects were observed for cocoa flavour, acidity and astringency. The interaction between clones and countries (environment) were non-significant (P=0.05), although the P values were relatively low (varying between 0.06 and 0.34).

Introduction

The International Clonal Trial (ICT) was established as part of the CFC-funded project entitled "*Cocoa Germplasm Utilization and Conservation: a Global Approach*" (1998-2004). Multilocational clone trials were established in 8 cocoa-producing countries with 20 cocoa clones, supplied by intermediate quarantine centres. The aim of the ICT was to distribute and evaluate interesting new cocoa clones, selecting superior clonal varieties and assessing the stability of economically important traits including quality, yield and disease and pest resistance in a wide range of environments (years and sites).

There are only a few previous reports of studies on genotype x environment interaction for flavour traits. The genotypic influence on flavour has been well established (Clapperton et al. 1994a-d; Fowler 1996; Lockwood and Eskes 1996; Figueira et al. 1997) and it is accepted that different cocoa varieties differ in their specific fermentation requirements. Fermentation and drying of cocoa are considered very important factors affecting flavour and have accordingly received considerable research attention. However, the relative contribution of the growing environment and processing-location-specific practices on flavour development is poorly understood and progress in this area has been limited since an objective and standardized method of evaluating flavour was lacking (Figueira et al. 1997).

Clapperton et al. (1994a) proposed a standardized method for carrying out micro-fermentations and flavour evaluations; these were subsequently optimized by Sukha et al. (2008). The optimized and validated protocol was used to delineate quantitative differences in flavour attributes among cocoa genotypes, examine crop year effects, as well as to determine the influence of environment on cocoa flavour profiles. Further studies by Sukha and Butler (2005) and Sukha (2008) have investigated the spectrum of flavour within genotypes and between origins but not in the detail and across as many different growing locations as presented in the current study. The micro-fermentation protocol initially proposed by Clapperton et al. (1994a) makes it possible to carry out individual tree evaluations for sensory traits and to substantiate the effect of genotypes on flavour components. In the current study, a similar method was adopted to evaluate flavour of samples coming from eight different sites and harvested in two different years.

The stability of cocoa flavour and astringency of several genotypes between Brazil and Malaysia has been shown (Figueira et al. 1997). However, other studies have demonstrated an effect of crop year on sensory results (Sukha 2008).

Materials and methods

Genotypes and environments tested

The ICT was established between 1999 and 2002 in eight different countries: Brazil, Côte d'Ivoire, Ecuador, Ghana, Malaysia, Papua New Guinea, Trinidad and Venezuela. Not all genotypes proposed in the ICT could be planted at all sites. The 11 more commonly planted genotypes were taken as the core for the sensory evaluation studies. These genotypes were AMAZ 15-15, EET 59, GU 255V, ICS 1, MAN 15-2, Mocorongo, PA 107, Playa Alta 2, SCA 6, UF 676 and VENC 4-4. In addition, some second priority clones were considered that were not present at a large number of sites, as well as local control clones that represented the locally important flavour characteristics. Beans were fermented in two years, i.e. during the main cropping cycles of 2006-07 and 2007-08. In total, 145 samples were analysed by the panels (Table 1). For the interaction studies between environment and clones, only clones tested for two years at a minimum of three sites were taken into account. This meant that Papua New Guinea (one single year) and Malaysia (very few samples) were eliminated from the analysis. The criteria above resulted in 80 samples being used for the environment x clone interaction studies.

Post-harvest handling and fermentation protocol

A standardized protocol for post-harvest handling and fermentation was developed. The main features of the protocol are described below.

- Pods were harvested one day before the start of fermentation.
- Each year, two fermentations were carried out, one at the beginning and one at the middle of the main cropping season.
- It was recommended to open the pods with wooden clubs to avoid cutting the beans.
- Samples of about 1200 g wet beans were used to obtain around 500 g of dry beans for each micro-fermentation.
- Clone samples were placed in nylon net bags for micro-fermentation at the start of fermentations in the central 30 cm of large fermentation boxes (minimum size 50 x 50 x 60 cm) filled with local cocoa beans.
- The fermentation was turned twice, at 48 hour intervals. The fermentation duration was 144 hours (6 days).

- After fermentation, the beans were air-dried in layers 4-5-beans-thick to ensure relatively slow drying.
- Drying should typically have taken 8-10 days, during which the beans were regularly stirred to encourage even drying.
- Drying was complete when the beans had reached about 7-8% moisture content.
- The two samples of 500 g dry beans were mixed after verifying that the two samples were of similar quality.
- On average, 1 kg of dry beans was sent to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in Montpellier, France, to assess for defects in the samples. CIRAD then sent the samples to the Guittard Chocolate Company in the USA for liquor making.
- Dry beans were roasted at 121°C for 25 minutes and cocoa liquors prepared at the Guittard Chocolate Company.

Liquor preparation

All cocoa bean samples were stored under cool room conditions (12-14°C) to curb insect infestation. Prior to roasting, beans were brought to room temperature for 24-48 hours and picked over by hand to remove all foreign materials (sticks, stones, clusters, shrivelled beans, etc.). The roasting oven used was a laboratory grade convection oven with mild air circulation. Ovens such as Binder Model FD23-L or equivalent provide rapid thermal recovery, uniform temperature profiles, and convenient digital thermal readouts. As with any oven, calibrations against known ovens need to be conducted to insure the appropriateness of the roast. These comparisons were originally held as part of the CFC/ICCO Fine Flavour Project in comparisons of the oven conditions at the Cocoa Research Unit, University of the West Indies (CRU-UWI), St. Augustine, Trinidad and Tobago and at Guittard Chocolate Company, Burlingame, California, and then later at Mars Chocolate North America, Elizabethtown, Pennsylvania.

For the purpose of the present study, a roasting condition of 121°C x 25 minutes was chosen. This condition develops sufficient chocolate/cocoa intensity for samples whose flavour profiles are dominant in this category, yet at the same time is appropriate for samples with dominant fruity, floral and more delicate flavours. Approximately 150-200 g of beans were roasted for each sample. Following roasting, the beans were cooled at ambient conditions, broken and winnowed in a John Gordon Catador CC-1 winnower; the clean nibs were then picked over by hand to remove all remaining lose and stuck shell. Liquor milling was conducted either in a 1-litre Capco Mortar mill 1099-PC or in multiple batches (combined after milling) in a Retsch RM200 mortar mill. Liquor milling was conducted to the point where all noticeable particles were milled out of the liquor to provide a smooth liquor feel in the mouth. Typical liquor milling times are 45–60 minutes at warm conditions (60°C max.). Liquor was partitioned into jars for panelling by CIRAD, CRU and Guittard Chocolate Company/Mars Chocolate North America. Liquors were stored under cool (12-14°C) conditions in tight-fitting sterile sample jars until all liquor samples were milled. Organoleptic profiling of the liquors by the different panels did not start until after all the liquors had been prepared and then shipped to their respective destinations to ensure that all the samples evaluated by the three panels would be identical.

Table 1. Cocoa liquor samples evaluated for sensory traits and used in the Multiple Factor Analysis

Clone	No. of samples								
	Brazil	**Côte d'Ivoire**	**Ecuador**	**Ghana**	**Malaysia**	**Papua New Guinea**	**Trinidad**	**Venezuela**	**Total**
MAN 15-2	2	2	2	2	2	1	2	2	15
EET 59	1	2	2	2		1	2	1	11
GU 255V	2	2		2		1	2	2	11
PA 107	2	2	2	1			2	2	11
AMAZ 15-15	1	1	2	2		1	2	1	10
Mocorongo	2	2		2	1			2	9
Playa Alta 2	2	1	2		2		2		9
SCA 6	2	2				1	2	2	9
ICS 1	1	2		2			2		7
IMC 47		2	1				2	2	7
VENC 4-4		1	1	2				2	6
CATIE 1000			2					2	4
IFC 5		2		2					4
UF 676		1	1	2					4
CCN 51	1		2						3
EET 103			2						2
GU 175P	1		1						2
ICS 43			2						2
PA 120	1		1						2
PBC 123					2				2
SC 10								2	2
SPEC 54-1	1		1						2
TSH 1076							2		2
C 15-161		1							1
GU 307	1								1
LCT-EEN 46	1								1
MO 20	1								1
NA 33	1								1
OC 61								1	1
T 79/501				1					1
TSA 644 x CCN 51	1								1
TSH 516	1								1
Total	**25**	**23**	**24**	**20**	**7**	**5**	**20**	**22**	**145**

Sensory evaluation protocols

Sensory evaluation of the cocoa liquors was carried out by three panels, one in Trinidad at the CRU-UWI (P1), one in France at CIRAD, Montpellier (P2) and one in the USA by the same person at the Guittard Chocolate Company for the first year and at Mars Chocolate North America for the second year (P3). The number of panellists, the scale of scoring, number and definition of descriptors varied in each panel. However, the same basic principles were used by all the three panels. Details are given in Tables 2 and 3.

Table 2. Panels that carried out sensory evaluations of 145 cocoa liquor samples

Panel	No. of panellists	Scale	Data utilized	No. of descriptors
P1	2	0-10	Mean of 2 replicates, reduced centre	9
P2	1	0-5	Mean of 1-3 replicates, reduced centre	17
P3	1	0-10	Mean of 5 replicates, reduced centre	44

Table 3. Comparison of flavour traits evaluated by the three panels

Panel	Descriptors
P1	Cocoa, acid, bitter, astringent, fruity, floral, nutty and raw/beany/green, off-flavours, other flavours
P2	Cocoa, acid, bitter, astringent, fresh fruit, brown fruit, floral, nutty, herbal, and off-flavours
P3	Cocoa, acid, bitter, astringent, fresh fruit, brown/mature fruit, floral, woody, spicy, nutty, mouldy, other off-flavours

The complete panellist training and sensory evaluation protocol used by Panel 1 in Trinidad is presented in Sukha (2008) and Sukha et al. (2008). As a summary, organoleptic analysis was done using two trained sensory panellists under controlled conditions with a hidden cocoa liquor reference from Ghana. Each sample was tasted blindly twice by each panellist according to a factorial design. Eight flavour attributes were assessed, viz. cocoa, acid, astringent, bitter, fruity, floral, nutty and raw/beany/green flavours. Additionally, unfermented and identifiable off-flavours such as smoky, hammy, mouldy were included in a section for "other" flavours. Panellists were also encouraged to identify any other ancillary flavours (such as caramel) or defects that were apparent in the cocoa liquors in a section for "other" flavours. The panellists scored flavour profiles of the cocoa liquors using open-ended 10-cm-line scales with a possible range of scores from 0 to 10 where the higher numbers denoted stronger flavour intensities.

In Panel 2 at CIRAD, France, the panellist scored the following flavours on a 0 to 5 point scale: cocoa, acid, bitter, astringent, fresh fruit, brown fruit, floral, nutty, herbal, and off-flavours. A minimum of one and a maximum of three replicate tastings were carried out.

Organoleptic panelling by Panel 3 (Guittard Chocolate/Mars North America) was done as follows. Prior to panelling, the liquor samples were brought to room temperature. Small plastic soufflé cups with tight-fitting lids were labelled with random 3-4 digit numbers to ensure that all organoleptic evaluations were blind. Five replicates of each sample were prepared and samples from flavour replicates were assembled into sets of 20-30 samples. For tasting, samples were randomly ordered and tasted with a timer marking 5 minutes ± 15 seconds between flavour evaluations. Samples were placed in a dry-bath incubator held sealed at 55-60°C for 20 minutes prior to organoleptic evaluation. After organoleptic evaluation, the following clearing procedure was used: a) the sample was expectorated; b) warm water rinse, expectorate rinse water; c) 1/8th-1/6th of a Carr Water Cracker Wafer was chewed and swallowed; d) warm water rinse, expectorate rinse water;

e) warm water rinse, swallow rinse water. Panel 3 used a total of 44 descriptors including cocoa flavour, four types of acidity (fruit, acetic, lactic, mineral), astringency, bitterness, seven types of fruity (berries, citrus, dark tree, tropical, dried, mature, over-ripe), six types of floral (woody, green, vegetative, earthy, herbal, mushroom), three types of woody (light, dark, resin), two types of nutty (nutty, nut skins), bark wood, caramel, roast degree, three types of spicy (tobacco, pepper, other), and ten types of off-flavours (mouldy, smoky, hammy, raw, leather, rotten fruit, putrid, manure, dirty, other). To be able to compare with the other panels, these descriptors were grouped as indicated in Table 3. Scores were given on a 0 to 10 scale, with higher notes indicating stronger flavour intensities. A total of five replicate tastings were performed on each sample.

Statistical analyses

Statistical analyses were performed using XLSTAT Version 2007.2. To homogenize the different scales and possible different uses of each scale, the data were centred and reduced by descriptor and by panel. The transformed scores analysed were therefore directly comparable between panels and descriptors.

Pearson's correlation coefficients were used to compare descriptors. The descriptors were classified according to their dissimilarities (Hierarchical classification according to the Ward method). Two descriptors that fell in the same cluster were considered to have described the samples in a similar way. Multiple Factor Analysis (MFA) was used to compare how panels noted samples. MFA is a generalization of Principal Component Analysis (PCA), which allows the same weight to be given to each panel, independently of the number of descriptors used.

Analysis of variance (ANOVA) of the data over two years and three panels was used to identify the effects of clone and environment on the traits studied. Only the clones tested by the three panels in at least three countries were used for ANOVA. The analysis identified whether the effects of (1) clones, (2) environment (country, soil, climate and post-harvest treatment), (3) year and (4) clone x environment interactions were significant. Repetitions were the scores from the three panels and there was no replicate for environment, clone or mode of sample preparation. Therefore, conclusions about interactions between environments and clones should be interpreted with care.

Results

Coherence between the standardized notations of the various panels

Seven descriptors were common to all panels. Linear correlation coefficients between similar descriptors between the three panels were positive (except for nutty) but non-significant (Table 4). These low linear correlation coefficients between similar terms used by the three panels suggest possible differences in vocabulary, flavour association and methods for scoring the samples for the same traits.

Table 4. Pearson's correlation coefficients between similar descriptors of different panels

Correlation	Cocoa	Acidity	Astringency	Bitterness	Fruity	Floral	Nutty
(P1 x P2)	0.25	0.39	0.08	0.26	0.29	0.49	0.09
(P1 x P3)	0.27	0.67	0.20	0.19	0.20	0.30	-0.21
(P2 x P3)	0.37	0.49	0.52	0.20	0.15	0.24	-0.16

Four clusters of descriptors were built by classification of all descriptors used by the panels. The repartitioning of similar descriptors in the clusters demonstrates the coherence of the panels (Table 5).

Table 5. Position of descriptors in clusters obtained by hierarchical classification

Cluster 1	Cocoa P1 P2 and P3
Cluster 2	Acidity and fruity P1 P2 and P3
Cluster 3	Bitterness and astringency P1 P2 and P3
Cluster 4	Floral P1 P2 and P3

The majority of the common descriptors for the three panels belong to the same cluster, indicating that they described the traits in a relatively similar manner. There was a link between acidity and fruity as well as between bitterness and astringency. For the nutty trait, the three panels were not coherent. This descriptor was eliminated from the study due to its large variability. ANOVA was applied to the scores given by the panels for the six remaining descriptors. The differences between panels increased the error but the relative coherence in the descriptors showed the most important effects.

In order to study in more detail the reasons of disagreement between panels, a Multiple Factor Analysis (MFA) was carried out. The individuals (samples) were described by several groups of variables (group = panel; variables = descriptors). The same weight was given to each panel, whatever the number of descriptors. This allowed for analysis of the relationship between the panels and the first axes of the MFA. The first axis shows variability that is in common between Panels 1 and 3, but has little in common with Panel 2 (Figure 1). Axis 2 is specific for Panel 2, axis 3 is common to Panels 2 and 3, whereas axis 4 is common to the three panels. Therefore, there are common factors between the panels but not always between the three panels at the same time. There appears to be more agreement in the description of samples between Panels 1 and 3 than with Panel 2.

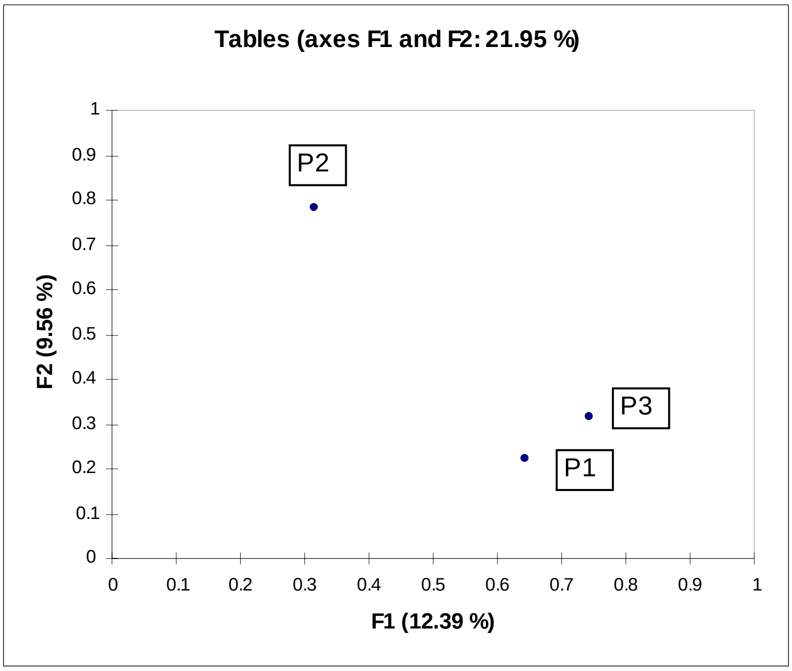

Figure 1. Representation of panels on the two first axes of MFA.

Figure 2 shows clustering of the sensorial descriptors on the two first axes of the MFA. Cluster 1 represents cocoa flavour and for some of the panels also nutty flavour. In Cluster 2 one finds fruity and acid traits. Cluster 3 represents bitterness and astringency, and Cluster 4 represents the floral flavour trait.

The first axis of the MFA which explains the largest variation between the cocoa samples shows a separation between the acid and fruity flavours on one side, and the cocoa flavour on the other side. Therefore, the samples which were acid and fruity showed in general less intense cocoa flavour. On the second axis, the samples that were bitter and astringent were opposite to those with more cocoa flavour. The variation for the floral flavour trait (on axis 3) appears to be independent from the other descriptors. On axis 4, the fruity cocoa samples were opposite to the bitter samples.

For Panels 1 and 2, the axis explaining most of the variability is strongly correlated with the axis 1 of the MFA: separation between acid, fruity and cocoa flavours. For Panel 2, the axis of highest variability (correlated with axis 2 of the MFA) separated bitterness and astringency from cocoa flavour. Panels 1 and 3 were more sensitive to the separation of samples with strong fruity and acid traits from those strong in cocoa flavour. On the other hand, for Panel 2 the main variability separated bitter and astringent samples from those rich in cocoa flavour. This could explain the low coefficients of correlation between panels, especially for the cocoa flavour trait.

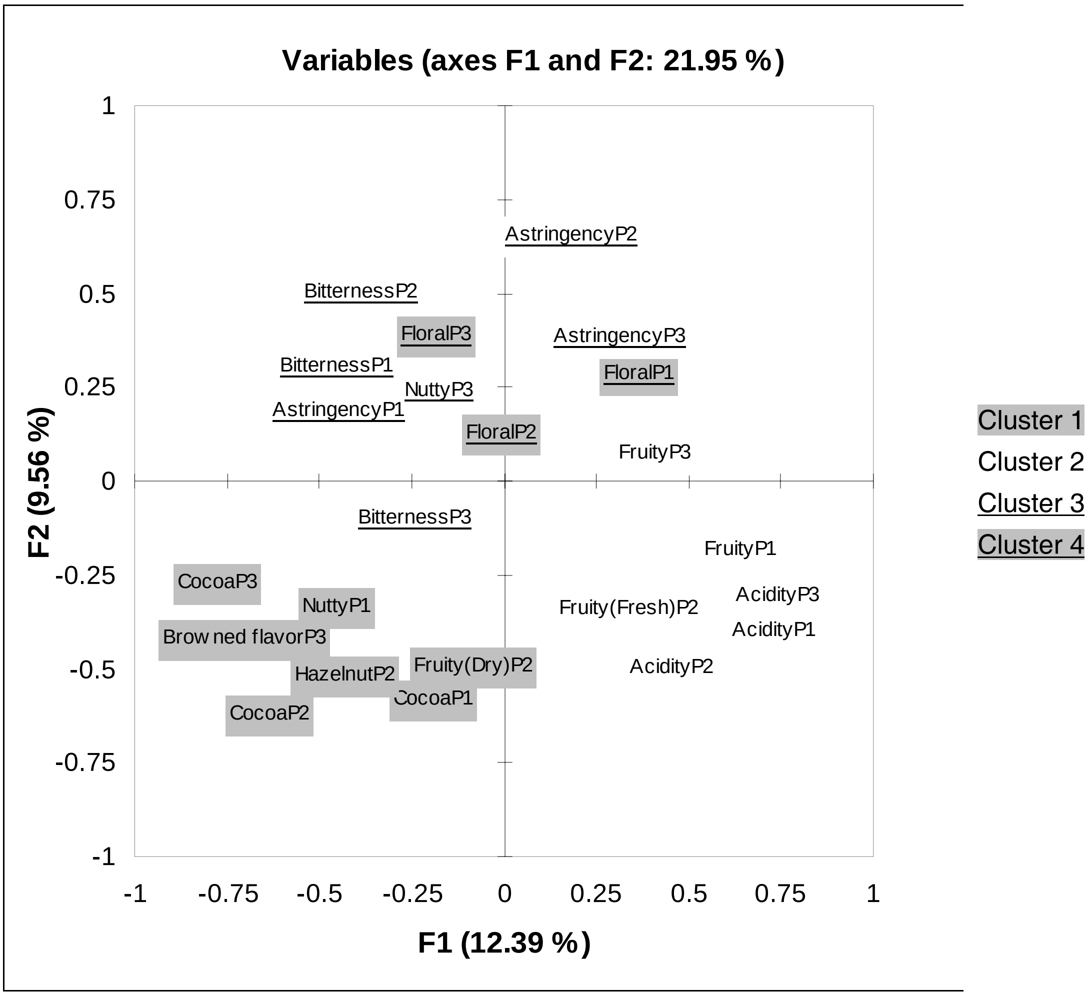

Figure 2. Representation of descriptors and clusters of descriptors on the two first axes of MFA.

Variability of flavour profiles: clones and country effects

The ANOVA results for the data compiled from the three panels are shown in Table 6. For most flavour traits (excluding floral), the country effect is more significant than the clone effect. In general, the interaction appears to be inferior to the main effects. An important effect of the year was observed on cocoa flavour, acidity and astringency. Because of the year effect, separate analyses were carried out for each of the two years (Table 7) and significant effects differed between the two years. In particular, the effect of country differed between the two years.

Table 6. Probability associated with Fisher statistics (F)

Source	Pr>F					
	Cocoa	**Acidity**	**Bitterness**	**Astringency**	**Fruity**	**Floral**
Clone	0.72	0.48	0.11	0.04	0.87	0.01
Environment	0.01	0.00	0.31	0.01	0.26	0.73
Year	0.00	<0.0001	0.25	<0.0001	0.82	0.50
Clone x environment	0.07	0.06	0.10	0.10	0.34	0.07

Table 7. Probability associated with Fisher statistics (F)

2006-2007	Pr>F					
Source	**Cocoa**	**Acidity**	**Bitterness**	**Astringency**	**Fruity**	**Floral**
Clone	0.34	0.29	0.35	0.77	0.98	0.00
Environment	<0.0001	0.00	0.16	0.01	0.36	0.46
Clone x environment	0.14	0.54	0.01	0.82	0.19	0.00

2007-2008	Pr>F					
Source	**Cocoa**	**Acidity**	**Bitterness**	**Astringency**	**Fruity**	**Floral**
Clone	0.51	0.05	0.20	0.09	0.18	<0.0001
Environment	0.11	0.00	0.48	0.31	0.12	0.02
Clone x environment	0.15	0.01	0.39	0.38	0.54	0.07

It was not possible to analyse all clone effects due to the non-orthogonal set-up of the data. However, there was a strong effect of clones on the floral trait over the two years. In 2007-08 there was a small country effect and in 2006-07 an interaction effect. So the floral trait was mainly due to a clone effect, with an interaction with the country effect that was sometimes significant. SCA 6, EET 59 and IMC 47 were generally the most floral clones (Table 8), whereas MAN 15-2, ICS 1 and Playa Alta 2 were the least floral clones. If one looks at the SCA 6 clone in particular, it is generally floral, but according to the years and countries, it can be averagely floral (with means near to 0) or very floral (means superior to 1).

Table 8. Newman-Keuls (SNK) analysis of the differences of floral mean between the clones with a confidence interval of 95%

Clone	Mean floral	Groups
SCA 6	0.95	a
EET 59	0.84	a
IMC 47	0.79	a
GU 255V	0.14	ab
PA 107	0.14	ab
AMAZ 15-15	-0.24	ab
Mocorongo	-0.25	b
MAN 15-2	-0.35	b
ICS 1	-0.36	b
Playa Alta 2	-0.47	b

Means followed by the same letters are not significantly different

Acidity appeared to be mainly related to a country effect, which could be an environmental or post-harvest effect. There was also a year effect, with higher acidity being observed in 2006-07 than in 2007-08 (Table 9). There was a general decrease in acidity over the two years, however this varied according to the country of origin. The decrease was especially pronounced for Ghana, Côte d'Ivoire and Venezuela. The average acidity was quite stable for Brazil and Trinidad, and increased slightly for Ecuador in the second year. Trinidad, Côte d'Ivoire and Ghana were among the most acid over the two years, with Brazil being the least acid.

Table 9. Newman-Keuls (SNK) analysis of the differences of mean acidity per year between the environments with a confidence interval of 95%

Country	Mean acidity 2006-07	Groups
Ghana	1.26	a
Papua New Guinea	0.97	a
Venezuela	0.76	a
Côte d'Ivoire	0.75	a
Trinidad	0.50	a
Ecuador	-0.41	ab
Brazil	-0.46	b

Country	Mean acidity 2007-08	Groups
Trinidad	0.22	a
Ecuador	0.07	a
Côte d'Ivoire	-0.32	a
Ghana	-0.47	a
Brazil	-0.63	ab
Venezuela	-0.91	b

Means followed by the same letters are not significantly different

Discussion and conclusions

Despite the limitations encountered in this study in generating and handling data from three different taste panels, the importance of the work should not be overlooked. This study represents the first attempt to investigate the spectrum of flavour within a diverse set of cocoa genotypes and between more diverse origins than had been previously attempted by any other group. The task to ensure that standard protocols were adhered to in the micro-fermentations and liquor sample preparation was an immense undertaking.

Sensory evaluation of cocoa liquors should follow a common set of principles in training, with reference liquors used to define and calibrate the vocabulary that ultimately describes a liquor sample. These principles were used in training the three panels used in this study. What differed between the three panels was the range of some of the descriptors used for some of the associations with the vocabulary, as well as the scale used to quantify the intensity of the flavours perceived.

The panellist associations for the sensory descriptors used to describe liquors are unique to the individuals in the taste panels. They are based on a mental library of flavours each panellist possesses which are defined by the taste experiences of the individuals. Training allows homogeneity of descriptors and references in a panel. This fundamental principle in flavour evaluation could account for the differences between the three taste panels and the similarities between the scores from Panel 1 and Panel 3. Individuals in these two panels especially have shared common training with similar flavour reference liquors, whilst the reference liquors used by Panel 2 for training were not shared with Panels 1 and 3.

What is also important to note is that despite some differences in the vocabulary used by the three panels, in many instances these were expansions of a more generic flavour descriptor. Taking Panel 3 for example, there were seven additional descriptors to add dimension to the more generic term “fruity”. Clustering the common descriptors for the three panels was an effective way of demonstrating that the panels described the flavour traits in a relatively similar manner.

The main results using the transformed data from the three panels were that floral flavour appeared to be mainly due to a clone effect. A similar dominant clonal effect for floral flavour was observed by Sukha (2008) which superseded the combined effects of growing and processing environments in some instances. The stability of the floral clonal effect has been linked to terpenes (Ziegleder 1990; Pino and Roncal 1992; Biehl and Voigt 1999). Terpenes are widespread in many plants as constituents of essential oils and are hydrocarbons; however, oxygen-containing compounds such as alcohols, aldehydes or ketones (terpenoids) are also found. According to Ziegleder (1990), linalool is the major terpene component in cocoa that contributes markedly to flowery-type flavours. Linalool is a product of biosynthesis in the maternally derived testa and cotyledon tissue and its generation depends in principle on plant varieties, cultivation and fermentation conditions (Ziegleder 1990). In cocoa pulp, terpenes exist in glycosidic-bound forms and are hydrolyzed during fermentation and drying due to enzymatic processes, temperature and acidity. Therefore, according to Ziegleder (1990), the maximum level of free linalool is found in raw cocoa beans as a biosynthesis product in testa and cotyledon tissue.

An effect of year was observed by the three panels on cocoa flavour, acidity and astringency. This global effect of the year over all countries could have been primarily due to a general climatic effect, especially during cocoa drying which could in turn affect drying rate, the cessation of cocoa fermentation during the initial stages of drying and drying time since all samples were naturally sun-dried. Other more secondary reasons for this effect could be due to an effect of the preparation of the liquors or to the evolution in the scoring of these flavours by the three panels in their respective evaluations.

Links between acidity and fruity flavours as well as between bitterness and astringency were also clearly identified by the three panels. Also acidity appeared to be mainly related to a country effect, which could be an environmental or post-harvest effect.

According to Biehl and Voigt (1999), the low amounts of organic acids (ascorbic, citric, malic, tartaric, oxalic and phosphoric) present in the pulp tissue do not contribute to the acidity of raw cocoa. Instead, acidification occurs during fermentation from the action of acetic and lactic acid bacteria producing acetic and lactic acid that infiltrates into the cotyledons of the bean. Lactic acid is produced from the pulp sugars, whereas acetic acid is a product of the oxidation of ethanol via the fermentation action of yeasts (Schwan and

Wheals 2004). Cotyledon acidification initiates a two-step proteolytic digestion of the major storage proteins which results in a mixture of amino-acids and oligopeptides that represent essential aroma precursors (Biehl et al. 1994; Biehl and Voigt 1999).

Residual acidity in liquors is also a function of drying rate. Previous work has linked drying rate to the acidic characteristics of cocoa (Jinap and Dimick 1990; Jinap 1994; Jinap and Thien 1994; Bonaparte et al. 1998) and the residual acetic and lactic acids in the bean have been strongly implicated as the major cause for acidic taste.

Fruity flavours on the other hand are based on the presence of esters derived from organic acids and alcohols which are themselves derived from sugar metabolism of the pulp. These esters enter the cotyledon tissue following the entrance of acetic acid and become associated with the fat present in the cotyledon.

With respect to bitterness and astringency, purine alkaloids (mainly theobromine and caffeine) have long been linked to bitterness in cocoa (Bonvehí and Coll 1997; Matissek 1997; Biehl and Voigt 1999). More recently Rotzoll et al. (2006) and Stoll et al. (2006) have shown that γ-aminobutyric acid (GABA), a non-protein amino-acid and a well-known plant stress metabolite, can also be linked to bitterness in cocoa. Purines are present as storage compounds in the cotyledon tissue, whilst GABA is actively synthesized in the first days of fermentation by the cotyledon tissue.

Astringency in unfermented cocoa is due to the tanning effect of mono- and oligomeric polyhydroxyphenols (mainly flavan-3-ols) which are stored in the vacuoles of specialized cells in seed cotyledons (Biehl and Voigt 1999). According to Lockwood and Eskes (1996), cotyledon colour is one of the few morphological characteristics in cocoa which is known to show simple (Mendelian) inheritance. Quantitative variation in anthocyanin contents gives shades of purple, even in true Criollo genotypes. Therefore the presence and linkage between bitterness and astringency to both clone and, to a greater extent, the degree of fermentation is clear.

This study has been able to further demonstrate the strong clonal effect on floral over diverse processing and growing environments (Sukha and Butler 2005; Sukha 2008). Despite limitations, the three sensory panels were able to identify strong year effects on certain flavours as well as correlations between certain flavours in clones that are stable over diverse processing and growing environments. It should be noted that the effects of clones and countries would probably have been more precise if there was less background "noise" in the dataset due to the incoherence of the panels.

What is needed therefore to further expand the knowledge base on the impact of *"terroir"* on cocoa flavour is more years of data and more repetitions of samples with diverse flavour attributes, as well as appropriately replicated sensory panel data that would allow for individual sensory panel analyses. The latter was not possible in this study due to inconsistent replications of tasting between panels.

Nevertheless the findings from this study are potentially very important from a marketing perspective since they provide further evidence to establish the applicability of the concept of *"terroir"* to cocoa.

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Analysis of physiological data from the International Clone Trial (ICT) at the University of Reading

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Abstract

A range of physiological parameters (canopy light transmission, canopy shape, leaf size, flowering and flushing intensity) were measured in the International Clone Trial, typically over the course of two years. Data were collected from six locations, these being: Brazil, Ecuador, Trinidad, Venezuela, Côte d'Ivoire and Ghana. Canopy shape varied significantly between clones, although it showed little variation between locations. Genotypic variation in leaf size was differentially affected by the growth location; such differences appeared to underlie a genotype by environment interaction in relation to canopy light transmission. Flushing data were recorded at monthly intervals over the course of a year. Within each location, a significant interaction was observed between genotype and time of year, suggesting that some genotypes respond to a greater extent than others to environmental stimuli. A similar interaction was observed for flowering data, where significant correlations were found between flowering intensity and temperature in Brazil and flowering intensity and rainfall in Côte d'Ivoire. The results demonstrate the need for local evaluation of cocoa clones and also suggest that the management practices for particular planting material may need to be fine-tuned to the location in which they are cultivated.

Introduction

Various studies have demonstrated genotypic variability in cocoa for a range of physiological traits. These include photosynthetic parameters (Baligar et al. 2008; Daymond et al. 2011) and canopy traits (Yapp and Hadley 1994; Daymond and Hadley 2002). From controlled environment studies on a limited range of cocoa germplasm evidence has been found of differential effects of temperature on growth, photosynthetic efficiency, canopy development and fruit development of contrasting cocoa genotypes (Daymond and Hadley 2004, 2008). However, little work has been conducted on the stability of physiological traits in cocoa under field conditions. The International Clone Trial (ICT) established in the CFC/ICCO/Bioversity project thus provides an opportunity to examine physiological traits in cocoa across a range of geographical locations.

The specific aims were to estimate the extent of variability in physiological traits across the clones used in the ICT and to assess the stability of such traits within and across sites, hence determining whether some clones are more susceptible to environmental stimuli than others.

Methodology

The design of the ICT is described by Eskes (2000). The trial has six blocks and eight replicate trees per block. A standardized set of working procedures were distributed to participating institutes early in the project. Physiological data were collected from the ICT at six locations, these being: Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura

Cacaueira (CEPEC/CEPLAC), Brazil; Instituto Nacional de Investigaciones Agropecuarias (INIAP), Ecuador; Ministry of Agriculture, Land and Marine Resources (MALMR), Trinidad; Instituto Nacional de Investigaciones Agropecuarias (INIA), Venezuela; Cocoa Research Institute of Ghana (CRIG) and Centre National de Recherches Agronomiques (CNRA), Côte d'Ivoire. The data collected at each site are summarized in Table 1.

Table 1. Summary of data collected at each site where the CFC International Clone Trial is located. Data refer to the number of times each parameter was measured.

Institute, Country	Flowering intensity	Flushing intensity	Leaves per flush	Leaf size	Canopy shape	Light interception
CEPEC, Brazil	13	13	1	2	1	1
INIAP, Ecuador	10	10	-	-	1	-
MALMR, Trinidad	14	16	4	2	1	2
INIA, Venezuela	12	12	-	2	2	2
CRIG, Ghana	1	1	-	-	1	1
CNRA, Côte d'Ivoire	12	12	-	1	1	1

Canopy characteristics

Canopy shape was determined using an index of 1-5, where 1 represents a horizontal spreading canopy shape and 5 a vertical erect canopy shape. For light transmission, a 0-5 scale was used corresponding to 0, 5, 10, 20, 40 and >50% light transmission. These measurements were made at the end of the rainy season, before pruning in 2006-07. Leaf size was measured in terms of leaf length and width of all leaves on two flushes on two trees in all of the six blocks.

Flowering and flushing patterns

Flushing and flowering intensity were recorded on a monthly basis in five locations (Brazil, Côte d'Ivoire, Ecuador, Trinidad and Venezuela) over the course of 2007-08. For these data sets indices were used as summarized in Table 2.

Table 2. Indices used for recording flushing and flowering intensity

Index score	Flushing (%)	Flowering (no. of flowers)
0	0	0
1	20	1-5
2	40	6-15
3	60	16-50
4	80	51-150
5	100	>150

Statistical analysis

Parameters were initially analysed within a given country for the effect of clone and, where applicable, the effect of time of year using analysis of variance (ANOVA). Where parameters were measured in more than one country, two-way analysis of variance was used to analyse the effect of clone and country.

Results

Canopy shape

Canopy shape was recorded by six participating countries (Brazil, Ecuador, Ghana, Côte d'Ivoire, Trinidad and Venezuela). In an analysis of 11 clones common to the six sites, significant differences were found between clones (P<0.001) with SCA 6 having the most spreading and SPEC 54/1 the most erect canopy (Figure 1). No significant differences were observed between sites.

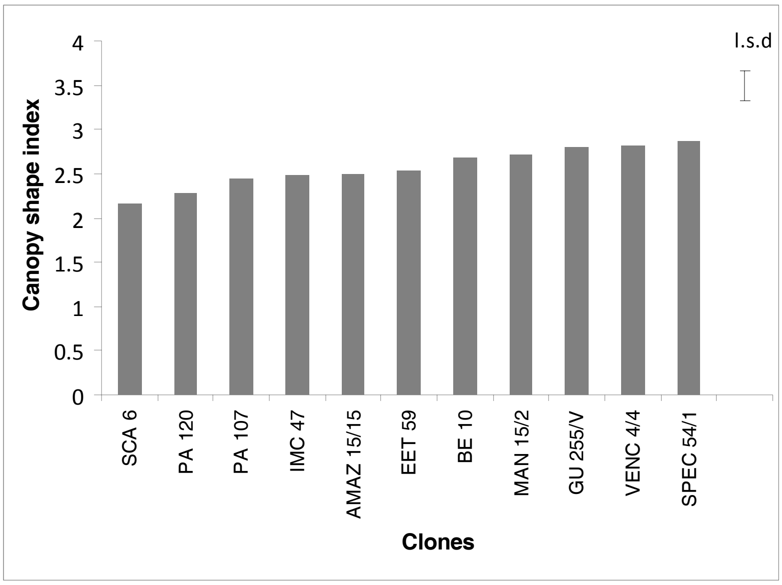

Figure 1. Canopy shape of 11 clones within the International Clone Trial (1 represents a spreading canopy and 5 an erect canopy). Values are the means across five different sites.

Light transmission

Light transmission was measured in four countries (Brazil, Ghana, Côte d'Ivoire and Trinidad) (Figure 2). An analysis of 11 clones common to the four sites demonstrated significant differences in light transmission between clones and across countries (P<0.01 in both cases). Furthermore, there was a significant clone x country interaction (P<0.05); for example, the clone IMC 47 exhibited a reasonably constant level of light transmission in all four sites, whereas SCA 6 exhibited a higher level of light transmission in Trinidad than at the other three sites.

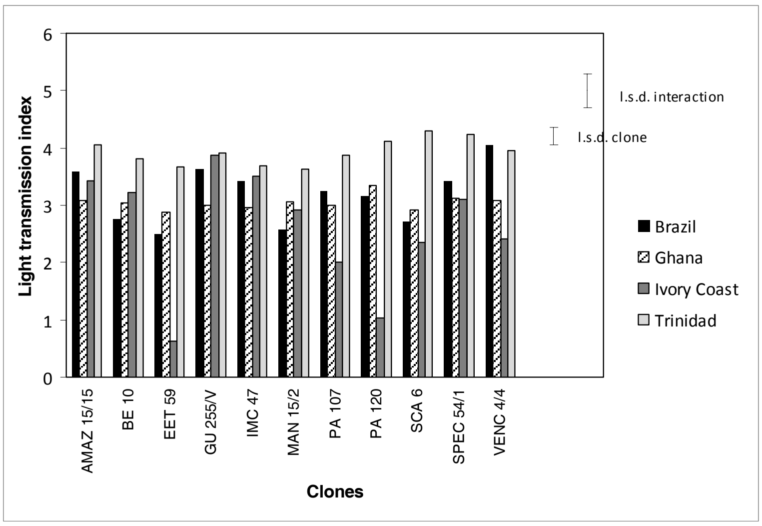

Figure 2. Light transmission of 11 clones within the International Clone Trial at four locations (1 represents a low level of light transmission; 5 a high level).

Leaves per flush

Number of leaves per flush was measured in Trinidad and in Brazil. The number of leaves varied significantly between clones (P<0.001) from an average of 4.1 for PA 107 to 5.2 to MXC 67. Whilst leaf number was consistently higher in Brazil (P<0.001) the magnitude of this difference was greater for some clones than others; hence the clone x country interaction was significant (P<0.05; Figure 3).

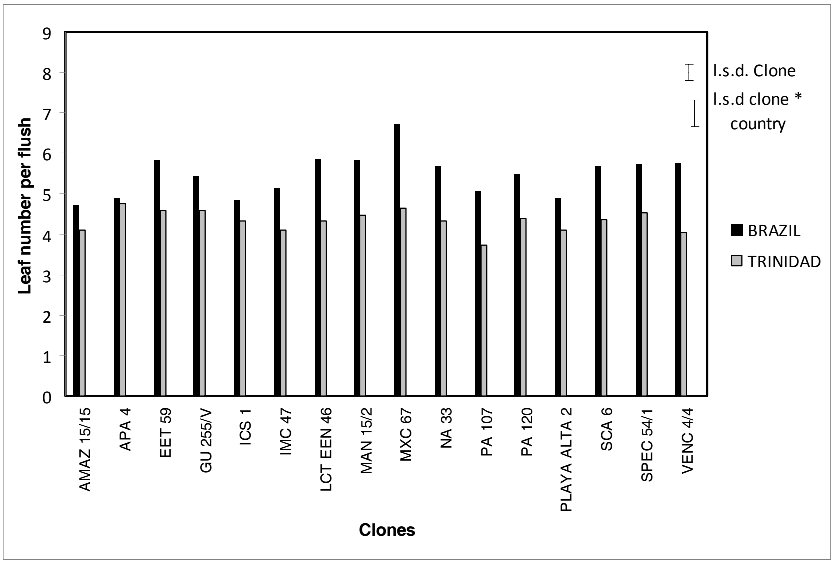

Figure 3. Leaf number per flush of 16 clones in the International Clone Trial in Brazil and Trinidad.

Leaf size

Leaf size (recorded as length and width) was measured at three locations: Brazil, Côte d'Ivoire and Trinidad; in Brazil and Trinidad leaves of different ages were distinguished. A comparison of leaf length of "leaf 1" (the youngest fully hardened leaf of a given flush) of 14 clones grown in Brazil and Trinidad is presented in Figure 4. Significant differences were observed between clones (P<0.001), the average length varying from 19.2 cm for SCA6 to 31.8 cm for PA 120. On average, leaves were 1.3 cm longer in Trinidad compared to Brazil (P<0.01). However, the magnitude of the difference between sites was considerably different between clones; hence there was a significant interaction between clone and location (P<0.001; Figure 4).

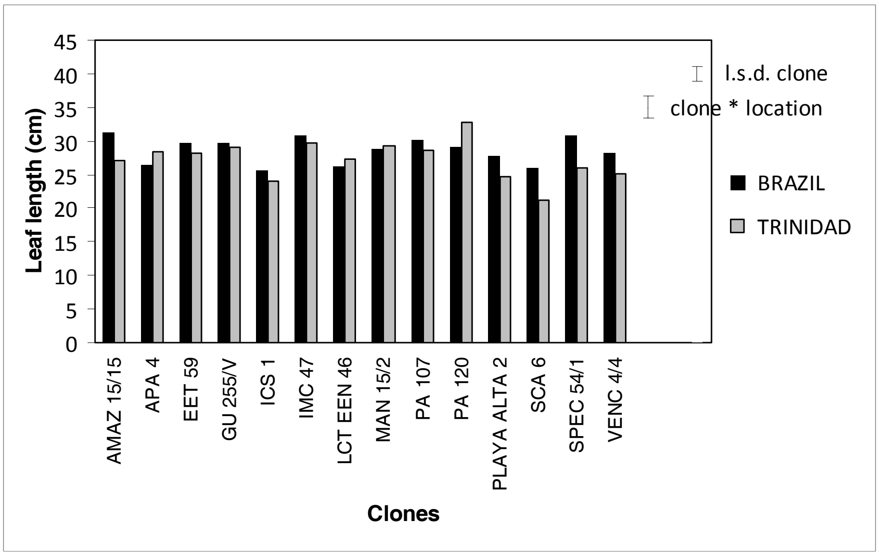

Figure 4. Leaf length of 14 clones in the International Clone Trial in Brazil and Trinidad.

Flushing

Flushing data were recorded monthly over the course of a year in Brazil, Venezuela, Trinidad and Côte d'Ivoire. Analyses were conducted within each country to test for the effect of clone and time of year (month). In all four countries there were significant differences between clones (P<0.001 in all cases), time of year (P<0.001 in all cases) and the interaction between clone and time of year (P<0.001 in all cases). The latter observation may indicate that some genotypes respond to a greater extent than others to environmental stimuli over the course of a year. The seasonal flushing pattern of five clones is illustrated in Figure 5.

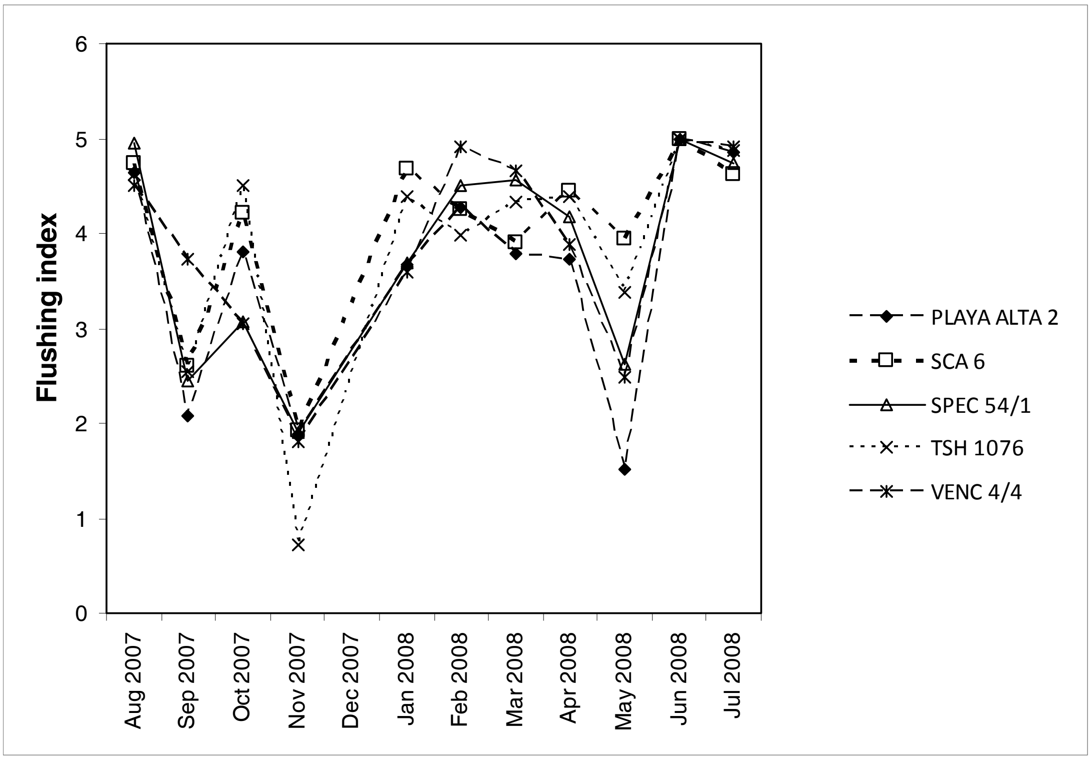

Figure 5. An example of the seasonal variation in flushing intensity of five clones in Trinidad from August 2007 to July 2008.

Flowering

Flowering intensity was recorded monthly over the course of a year in Brazil, Venezuela, Trinidad and Côte d'Ivoire. Analyses were conducted within each country to test for the effect of clone and time of year (month). In all four countries there were significant differences between clones (P<0.001 in all cases) and time of year (P<0.001 in all cases). Furthermore, the interaction between clone and time of year was also significant (P<0.001 in all cases), reflecting that particular genotypes show greater seasonal variability in their flower numbers compared with others. An example of the flowering pattern of five clones over the course of a year in Brazil is given in Figure 6. When looking at factors underlying seasonal variation in flowering within the ICT in Brazil, temperature was found to be highly correlated with flowering intensity (flower numbers were suppressed at cooler temperatures). A comparison of regression revealed differences amongst clones in their response to temperature (see Figure 7 for an example of two clones). At the Côte d'Ivoire location a positive correlation was found between rainfall and flowering intensity (Figure 8).

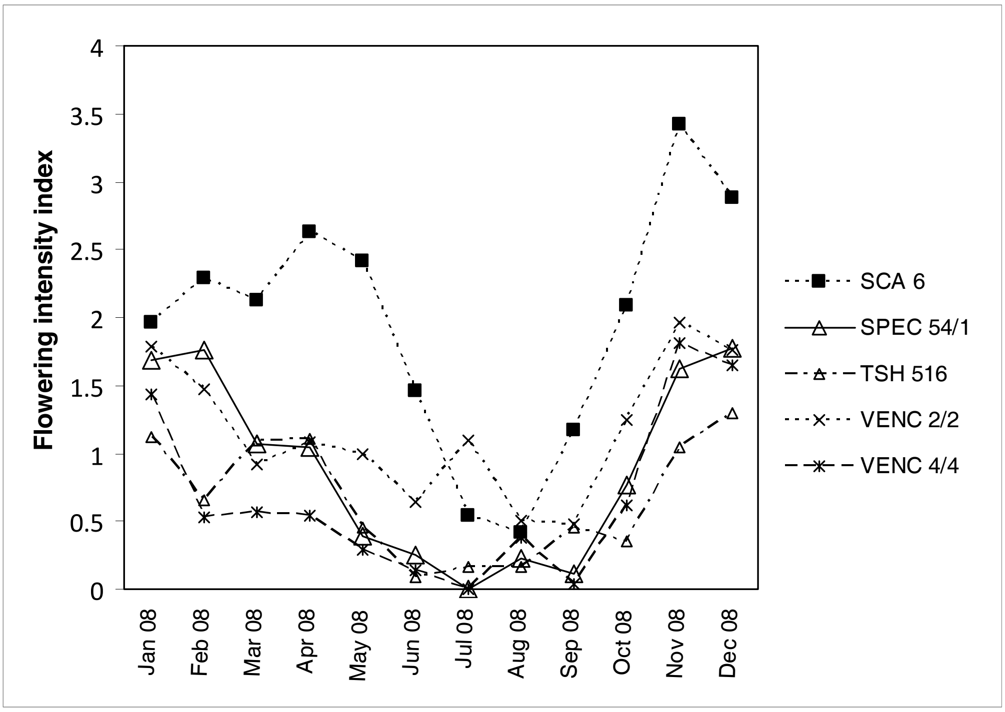

Figure 6. An example of the seasonal variation in flowering intensity of five clones in Brazil between January and December 2008.

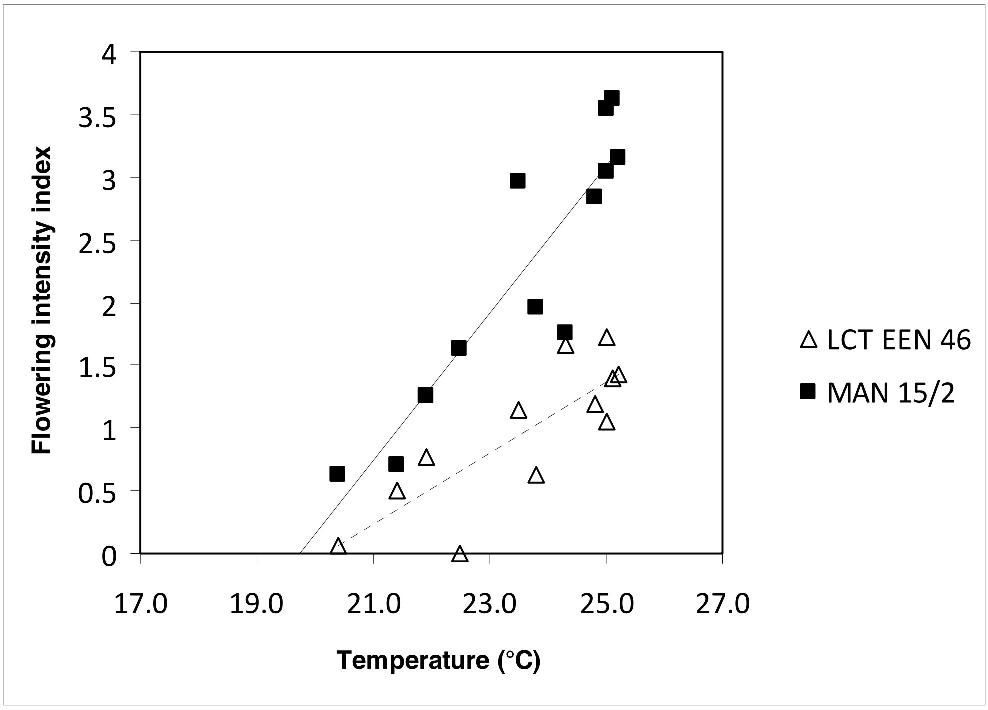

Figure 7. The response of flowering intensity of two contrasting clones to monthly mean temperature in Bahia, Brazil.

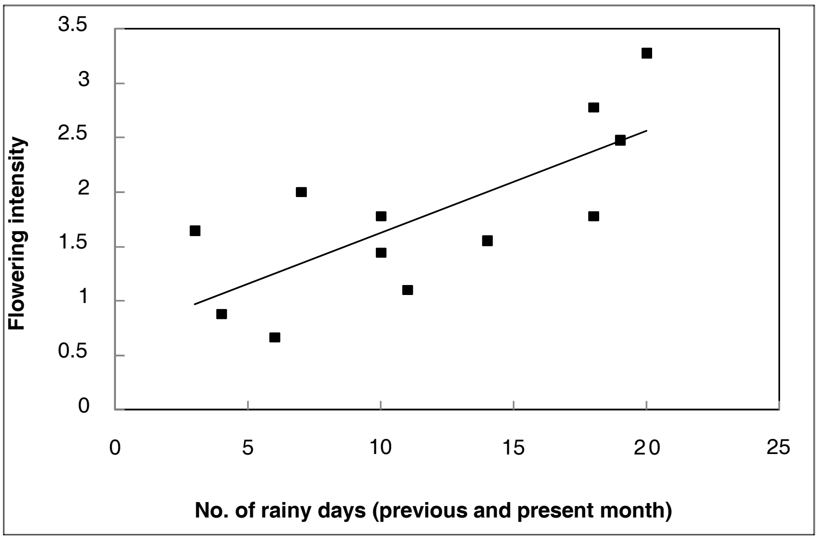

Figure 8. Relationship between rainfall (number of rainy days over two months) and flowering intensity in the International Clone Trial, CNRA, Côte d'Ivoire.

Discussion and conclusions

The results demonstrate a high degree of genotypic variation in canopy shape and the amount of light transmitted through the canopy, confirming previous studies showing genotypic differences in canopy architecture (Yapp and Hadley 1994; Daymond et al. 2002). A unique aspect of the International Clone Trial is that for the first time the performance of a set of clones has been compared in different cocoa-growing countries, allowing the stability of traits to be assessed. It is implicit in the results that some clones show greater plasticity than others in relation to individual leaf and flush size, which in turn are factors that impact on the amount of light intercepted by the canopy. This has a potential impact on the management of cocoa material in different locations. For example, light interception by the canopy is a factor that influences optimal planting density; if the canopy architecture of particular clones is differentially affected by local environmental conditions, then this may in turn impact on what planting density is most appropriate.

The data collected on flushing over the course of a year at four different locations demonstrate genotypic differences in flushing intensity and hence the rate of canopy expansion. Flushing is stimulated by environmental factors such as temperature and water availability (Alvim 1977). The fact that the magnitude of differences in flushing (and hence the rate of canopy expansion) between clones varied over time in each of the locations suggests that some clones may be more sensitive than others to such stimuli. Experiments under greenhouse conditions using selected clones from the ICT have also shown that the rate of canopy expansion of different clones varies in response to temperature (Daymond and Hadley 2008).

Flowering intensity varied across the season in four different locations to a greater extent for some genotypes than others. This is indicative of both genotypic differences in flower numbers but also may indicate differential sensitivity of genotypes to environmental stimuli. The latter hypothesis is strengthened by the analyses of the data collected at CEPEC, Brazil where differential responses to temperature were observed. Flowering is also known to be affected by other factors, including temperature and light intensity (Hurd and Cunningham 1961; Sale 1970). The implication of the results is that clones that are more sensitive to particular environmental stresses will show greater variability in their cropping pattern in those areas where such stresses are prevalent.

Overall, it may be concluded that:

1. Some clones showed greater variability than others both within and across sites with respect to physiological and phenological parameters that impact on yield potential and cropping patterns. The results therefore highlight the need for local evaluation of material in selection and breeding trials.
2. The recommended husbandry practices associated with a given clone (e.g. optimal planting and pruning practices) may vary between growing regions.

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RESISTANCE STUDIES

Progress obtained in Côte d'Ivoire on mirid resistance studies

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Abstract

The development and use of resistant cocoa varieties is no doubt a major component of the integrated management of cocoa pests and diseases. However, breeding cocoa for resistance to cocoa pests, especially mirids, has been confronted to the lack of appropriate screening methods to identify sources of resistance. During the past five years, the implementation of the CFC/ICCO/Bioversity project on *Cocoa Productivity and Quality improvement: a Participatory Approach* has enabled to develop a new screening method for cocoa tolerance to mirids. This method is based on the reaction of the plant to the fungi associated to dieback due to mirid feeding lesions, after identification of the fungus species involved. The fungi species associated to mirid feeding lesions were isolated from cocoa twigs and pods naturally attacked in the field and were identified, the most predominant being *Lasiodiplodia theobromae*. The tolerance test consists in the evaluation of the level of natural infection of the cocoa genotypes to be evaluated by *Lasiodiplodia* after mechanical injury in the field or in the budwood garden. Results have shown that susceptibility of the cocoa tree to *Lasiodiplodia* varies from one genotype to another. Hence, the size of the lesions caused by *Lasiodiplodia* could be a good indicator of resistance to that pathogen and therefore an indicator of tolerance to mirid. However, correlation between two series of tests performed using the same clones was positive ($r=0.43$, $P=0.23$) but not significant, suggesting that the method need to be refined. The CFC/ICCO/Bioversity project also allowed investigating various components of cocoa resistance to mirids using laboratory and field screening methods. Results have revealed significant positive correlation ($r=0.97$, $P=0.005$) between field damage and antixenosis in the laboratory, indicating that antixenosis is involved in the level of damage observed in the field. Moreover, selected cocoa clones and hybrids have been screened for resistance/tolerance to *Sahlbergella singularis*, using the available screening methods. The major achievements are presented here.

Introduction

The cocoa mirids, also known as capsids, are the most economically important pests of cocoa in West and Central Africa. Of the four species causing damage to cocoa in Côte d'Ivoire, *Sahlbergella singularis* (Haglund) is the most predominant. Both adult and immature stages of mirids feed on every part of the plant except the leaves and the roots (Williams 1953; Taylor 1954; Kay 1961; Gibbs and Pickett 1966; Braudeau 1969; Kumar and Ansari 1974). It has been reported that the feeding wounds are subsequently invaded by a pathogenic fungus, *Calonectria rigidiuscula* (Berk. and Br.) (Crowdy 1947). This fungal attack results in cankering or bark roughening, destruction of the flower cushions and a severe dieback of twigs and branches.

For many years, breeding cocoa for mirid resistance has been confronted to the lack of an appropriate screening method to identify sources of resistance. Indeed, mirid resistance studies in cocoa have been conducted by several researchers including Bruneau de Miré and Lotodé (1974), Decazy and Lotodé (1975), Decazy and Coulibaly (1982), Nguyen-Ban (1994), Sounigo et al. (2003), N'Guessan et al. (2005, 2006) and Babin et al. (2004). However, screening was mostly based on assessment of field damage. Recently, a few works have focused on the mechanisms of mirid resistance in cocoa (N'Guessan et al. 2008), with regard to the well-known general mechanisms of insect resistance (Painter 1951; Kogan and Ortman 1978). Antixenosis tests based on attractiveness of fragments of cocoa twigs have been useful in selecting resistant cocoa genotypes in the laboratory (Nguyen-Ban 1994; N'Guessan et al. 2008; Anikwe et al. 2009). Attempts to develop antibiosis tests on pods and twigs as well as tolerance tests on twigs in budwood garden and in the field gave promising results but showed some limits. N'Guessan et al. (2006) mentioned a number of constraints, including: the fragility of mirids, making manipulation difficult; the problem of availability of mirids for tests because of the lack of a good rearing technique; mirid predation by ants in cages during field tests; and the need to wait for flushes for mirid resistance tests coupled with non-equal flushing patterns for different genotypes.

Knowing that the dieback due to mirid attacks is largely attributed to the effect of fungi associated to the mirid feeding lesions, further studies have taken into consideration the development of a tolerance test based on the reaction of the cocoa tree to the fungi associated to dieback. Some important results have been obtained through the CFC/ICCO/Bioversity project which has enabled to develop a screening method for tolerance to mirids, based on mechanical injury of cocoa twigs and natural infection of the wounds by *Lasiodiplodia theobromae,* the main fungal species associated to mirid damage lesions. In addition, selected cocoa clones and hybrids have been screened for resistance/tolerance to *Sahlbergella singularis*, using the available screening methods.

Development of a screening test for tolerance to cocoa mirids based on reaction to Lasiodiplodia theobromae

Identification of the fungi associated to mirid damage

In order to identify and use the most predominant fungus species associated to mirid damage, chupons and pods attacked by mirids were collected throughout the cocoa-growing regions of Côte d'Ivoire and brought to the laboratory. Plant materials were collected from Daloa, Soubré, Grand Zattry, Oumé, Gagnoa and Yamoussoukro. Mirid species responsible for the damage lesions on these materials were *S. singularis, Distantiella theobromae* and *Bryocoropsis laticolli*. In the laboratory, all the samples were washed with tap water, and the lesions were then disinfected with 95% ethylic alcohol. The bark was peeled using sterile scalpel. Two 1-cm-long fragments were sampled from the lesion near the fungal developing zone and placed in a 90-cm-diameter Petri dish containing an agar growth medium. Fragments of pods were sampled and processed similarly. The medium also contained four antibiotics: neomycin (0.1 g/l), penicillin (0.1 g/l), bacitracin (0.1 g/l) and aureomycin (0.025 g/l). After 4 days of incubation in the dark at 26°C, the different growing colonies of fungi were individually picked and placed in pea-based growth media containing the same four antibiotics. The fungal isolates obtained were purified and single-spore cultured without antibiotics. The different isolates of fungi obtained on the basis of macroscopic (mycelium aspect and colour) and microscopic (form of mycelium, aspect of conidia) characteristics were sent to Montpellier (France) for identification.

Based on the above characteristics, four groups of fungal isolates were identified. The first group was characterized by a light-brown aspect becoming dark. The mycelium is segmented, branched, and dark brown. The spores, made of two similar cells, are dark brown, ovoid and round-ended. This group was identified in Montpellier as *Lasiodiplodia theobromae*. The second group was also characterized by segmented and branched mycelium.

However, in this group, the mycelium was cotton-white, pinkish or yellow, flat or raised, with simple conidiophores. The macroconidiae are arched, sharp-ended and segmented. The microconidiae are unicellular, ovoid or oblong-shaped. This group was identified as *Fusarium* sp. The third group included fungal species such as *Trachysphaera fructigena, Colletotricum* sp. and *Verticillium* sp. The fourth group which was not identified was characterized by a slow growth and the lack of fructification.

Overall, *Lasiodiplodia theobromae* represented 47% of the fungi associated to mirid feeding lesions. *Fusarium* sp. accounted for 35%, the third group represented 10% and the remaining unidentified group accounted for 8%. *Lasiodiplodia theobromae* appeared therefore to be the most predominant fungus species involved in dieback due to mirid damage in cocoa.

Description of the mirid tolerance test based on *Lasiodiplodia* resistance

The tolerance test is based on the evaluation of the level of natural infection of the cocoa genotypes to be evaluated by *Lasiodiplodia* after mechanical injury in the field or in the budwood garden. A fine needle is used to wound ten semi-hardened shoots of the genotypes to be evaluated, making two punctures on each internode to simulate lesions caused by mirids. The shoots are left to natural infection by *Lasiodiplodia* for 6 weeks. After the 6-week-period, the shoots are cut off and brought to the laboratory. The shoots are then cut into fragments representing the internodes. The fragments are then split longitudinally to look for black stains on the inner side of the wounded areas. These marks are scored using a scale of 0 to 5 according to the size of the stain. The larger the size of the stain, the more susceptible the genotype. The presence of *Lasiodiplodia* in the stains was confirmed by isolation of this fungus in the mechanical wound.

Standardization of the mirid tolerance test based on *Lasiodiplodia* resistance

In order to determine the optimal duration of development of the *Lasiodiplodia*, which allows discriminating between the genotypes tested, the development of *Lasiodiplodia* in the mechanical lesions was evaluated at five different times, i.e. 2, 3, 4, 6 and 8 weeks after mechanical injury. The tests were conducted with five clones: NA 32, SCA 6, PA 150, ICS 1 and T 85/799. For each clone, at least 30 semi-hardened shoots were selected and mechanically wounded as described above. Four shoots of each clone were cut 2 weeks after mechanical injury and dissected by cutting and longitudinal splitting. The stains were rated as described above. The cutting of shoots, dissection and ratings of stains were repeated for the other times of development of *Lasiodiplodia* in the mechanical lesions (3, 4, 6 and 8 weeks after mechanical injury), four shoots being cut every time.

Furthermore, in order to determine the optimal period of growth of *Lasiodiplodia* after cutting the shoots, several time intervals of rating the lesions after cutting were evaluated. These were 0, 2, 4, 5, 7 and 8 days after cutting the twigs. The clones NA 32, SCA 6, PA 150, ICS 1 and T 85/799 were also used. Here again, at least 30 semi-hardened shoots were selected and mechanically wounded as described above. However, all the wounded shoots were cut and brought to the laboratory. Four shoots of each clone were dissected the same day. The dissection and scoring of four shoots per clone were repeated at five different time intervals after cutting the twigs (2, 4, 5, 7 and 8 days).

The results revealed significant (DF=4; F=33.6; P=0.0001) differences between the time of development of *Lasiodiplodia* in the mechanical lesions. The score increased with the time after mechanical injury (Table 1). The analysis of variance (ANOVA) performed on each time interval after injury showed that the best discrimination between the genotypes is obtained with 6 and 8 weeks after injury (Table 2). On the other hand, no significant (DF=5; F=1.7; P=0.11) differences were found between the time intervals of conservation of the twigs after cutting (Table 3). This suggested that keeping the twigs after cutting does not improve *Lasiodiplodia* development or the size of the stains. These results suggested that the mechanically-injured twigs should be cut 6 weeks later and rated the same day to have the best discrimination between the genotypes tested with regard to *Lasiodiplodia* infection.

Table 1. The effect of time on the development of *Lasiodiplodia theobromae* after mechanical injury of twigs in the field

Time after injury (weeks)	Rating score	Mean grouping
8	2.52	a
6	2.26	b
3	2.23	b
4	2.04	c
2	1.79	d

Scores followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Table 2. ANOVA results for time of development of *Lasiodiplodia theobromae* after mechanical injury to cocoa twigs

Time after injury (weeks)	DF	F	P
2	2.30	3	0.02
3	2.21	2.04	0.1
4	2.17	1.07	0.3
6	2.13	5.76	0.0008
8	2.08	5.67	0.001

Table 3. Effect of time of conservation of the mechanically-punctured twigs after cutting on the development of *Lasiodiplodia theobromae*

Time interval for rating the lesions after cutting the twigs (days)	Rating score	Mean grouping
7	2.30	a
4	2.21	a
8	2.17	a
5	2.13	a
0	2.09	a
2	2.08	a

Scores followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Application of the mirid tolerance test based on *Lasiodiplodia* resistance

Selected international clones were evaluated in two series using the tolerance test based on *Lasiodiplodia* resistance. A fine needle was used to wound ten semi-hardened shoots of each clone, making two punctures on each internode to simulate lesions caused by mirids as described above. The shoots were left to natural infestation by *Lasiodiplodia* for 6 weeks. After the 6-week-period, the shoots were cut off, brought to the laboratory and cut into fragments representing the internodes. The fragments were then split longitudinally to look for black stains on the inner side of the wounded areas. These marks were scored using a scale of 0 to 5 according to the size of the stain.

The results revealed significant differences between the clones in series 1 (DF=12; F=4.4; P=0.0001) and in series 2 (DF=11; F=13.9; P=0.0001) with regard to *Lasiodiplodia* infection. The susceptible clones IFC 5 and ICS 39 sustained high level *Lasiodiplodia* infection in the study (Table 4). Similarly, the resistant clones PA 120 and PA 150 had low *Lasiodiplodia* infection. However, there were some inconsistencies in the results. Indeed, clones such as Mocorongo sustained moderate level of *Lasiodiplodia* infection in series 1 and low level of infection in series 2. In addition, resistant clones such T 79/501 and T 60/887 ranked susceptible. Furthermore, correlation between the two series of tests was positive (r=0.43) but not significant (P=0.23), suggesting that the method needs to be refined. Nevertheless, results have shown that susceptibility of the cocoa tree to *Lasiodiplodia* varies from one genotype to another. Hence the size of the lesions caused by *Lasiodiplodia* could be a good indicator of resistance to that pathogen and therefore an indicator of tolerance to mirid.

Table 4. Behaviour of selected international clones vis-à-vis *Lasiodiplodia* infection after mechanical injury

Series 1		Series 2	
Clones	**Score**	**Clones**	**Score**
IFC 5	2.9 a	ICS 39	3.9 a
T 85/799	2.8 ab	T 79501	3.0 b
SCA 6	2.7 abc	LECTEEN 46	2.9 bc
T 79/501	2.7 abc	ICS 1	2.9 bc
ICS 39	2.6 abcd	IFC 5	2.9 bc
P 7	2.6 abcd	SCA 6	2.7 cd
Mocorongo	2.5 bcde	IMC 47	2.6 cde
ICS 1	2.5 bcde	CATIE 1000	2.5 def
PA 107	2.4 cde	GU 225V	2.5 def
IMC 47	2.3 def	PA 120	2.4 def
PA 150	2.2 ef	PA 107	2.3 ef
PA 120	2.2 ef	Mocorongo	2.2 f
EQX 3360-3	1.9 f	-	-

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Screening of project materials

Antixenosis resistance to mirid for selected cocoa clones and hybrids

The objectives of this study were to confirm mirid resistance in selected promising cocoa genotypes and to determine whether antixenosis was involved in the mechanism of resistance. The study also aimed at determining whether a resistant character is transmissible from the parents to the progenies. The study was conducted in 2006-07 at the research station of the Centre National de Recherches Agronomiques (CNRA) in Divo, Côte d'Ivoire, within the framework of the CFC/ICCO/Bioversity project. The methods applied follow the recommended Working Procedures for the CFC/ICCO/IPGRI project (Eskes et al. 2000), with slight modifications described hereafter.

Nine clones and ten hybrids were tested. The susceptibility of the clones to *S. singularis* has been shown in previous studies (Sounigo et al. 1993). Clones ICS 39, IFC 5, UF 667 were found to be susceptible whereas clones P 7, UPA 402, T 60/887, NA 32, T 79/501 and PA 150 were identified as promising for resistance to mirids because they sustained low long-term damage in the field. The ten hybrids were obtained from crosses made between promising clones (R x R), between susceptible clones (S x S), and between promising and susceptible clones (R x S) (Table 5). The hybrids and the parental clones were evaluated separately for attractiveness to mirids in the laboratory using a choice test. The clone IFC 372, a Catongo clone, was included as a susceptible control in the clone test.

Table 5. List of cocoa genotypes evaluated for antixenosis

Parents		Hybrid progenies	
IFC 372	Susceptible (S)	ICS 39 x IFC 5	Susceptible (S)
ICS 39		T 79/501 x IFC 5	R x S
IFC 5		IFC 5 x T 79/501	
UF 667		ICS 39 x T 60/887	
P 7	Resistant (R)	T 60/887 x ICS 39	
UPA 402		UF 667 x PA 150	
T 60/887		PA150 x UF 667	
NA 32		P 7 x T 60//887	Resistant (R)
T 79/501		T 79/501 x UPA 402	
PA 150		PA 150 x NA 32	

The experimental design was an incomplete block with 9 replicates, 18 blocks and 5 twig fragments per block for the parental clones and the hybrid progenies. Such a design allows the comparison of each genotype with the others within the same experimental set-up (Cochran and Cox 1957). Ten series were carried out for both the parents and the hybrid progenies in 2006 and 2007.

Healthy green twigs of young flushes obtained from the field were brought to the laboratory and cut into 6-cm sections. Five fragments of twigs with the same diameter and representing five different cocoa genotypes were placed end-to-end in 18 large Petri dishes (16 cm diameter x 2 cm height), according to the experimental design. One 4th instar mirid nymph that had been starved for 24 hours was placed in each Petri dish. The mirid nymphs were collected from the field the day before using them in the experiment. The insects were allowed to feed for 24 hours and the feeding lesions on the fragments of twigs were counted for each genotype in order to assess attractiveness of the different genotypes. Data were analysed using the GLM (General Linear Model) procedure of SAS (SAS Institute 1996).

Significant ($P<0.01$) differences were observed between the nine parental clones in 2006 and 2007 with regard to the number of mirid feeding lesions. Clones PA 150, T 79/501, NA 32, T 60/887 and UPA 402 were the least attractive in the laboratory (Table 6). These clones sustained between two and five lesions compared to the most attractive ones and the susceptible control that sustained between eight and ten lesions. The number of feeding lesions also varied significantly among the hybrids progenies during the two years. The hybrids PA 150 x NA 32, T 79/501 x UPA 402, and P 7 x T 60/887 were among the least attractive with numbers of lesions between two and three, compared to the most attractive hybrids having eight or nine lesions (Table 7). These least attractive hybrid progenies were obtained from crosses between promising parents.

Table 6. Numbers of feeding lesions of *S. singularis* nymphs on fragments of twigs of 10 cocoa clones

2006			2007		
Parent clones	**Feeding lesions**		**Parent clones**	**Feeding lesions**	
IFC 372	9.9	a	IFC 372	10.28	a
ICS 39	8.6	b	IFC 5	8.88	ab
IFC 5	7.8	b	ICS 39	8.36	b
UF 667	7.5	b	UF 667	7.44	bc
P 7	6.0	c	P 7	6.14	c
UPA 402	4.7	d	T 60/887	4.47	d
T 60/887	4.2	d	T 79/501	4.39	d
NA 32	4.2	d	UPA 402	4.26	d
T 79/501	4.1	d	NA 32	3.90	de
PA 150	2.8	e	PA 150	2.68	e

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Table 7. Numbers of feeding lesions of *S. singularis* nymphs on fragments of twigs of 10 cocoa hybrids

2006			2007		
Progenies	**Feeding lesions**		**Progenies**	**Feeding lesions**	
ICS 39 x IFC 5	8.4	a	ICS 39 x IFC 5	9.87	a
T 79/501 x IFC 5	4.6	b	IFC 5 x T 79/501	4.53	b
IFC 5 x T 79/501	4.6	b	ICS 39 x T 60/887	3.96	bc
ICS 39 x T 60/887	3.9	bc	UF 667 x PA 150	3.92	bc
T 60/887 x ICS 39	3.7	bcd	T 79/501 x IFC 5	3.68	bc
UF 667 x PA 150	3.7	bcd	PA 150 x UF 667	3.55	bc
PA 150 x UF 667	3.4	cde	P 7 x T 60/887	3.05	cd
P 7 x T 60//887	3.3	cde	T 60/887 x ICS 39	2.87	cd
T 79/501 x UPA 402	2.7	de	T 79/501 x UPA 402	2.57	cd
PA 150 x NA 32	2.4	e	PA 150 x NA 32	1.76	d

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test)

The results showed that the parents known to be susceptible were also ranked susceptible in the choice test, indicating that antixenosis or feeding preference may be related to the damage caused in the field. Moreover, hybrids obtained from crosses between susceptible parents ranked susceptible in the test. The hybrids obtained from crosses between promising clones sustained the lowest number of feeding lesions, suggesting that the antixenosis trait was transmitted from the parents to the progenies (Table 7). Overall the results suggested that antixenosis is involved in the mechanism of resistance of the genotypes tested and that this character is transmissible from the parents to the progenies.

The use of resistant varieties is an important and effective tool for pest control. Three mechanisms of plant defence to insect damage have been described (Painter 1951; Kogan 1982; Smith 1989). Although the causes of antixenosis have not been investigated in this study, previous research on other crops has attributed antixenosis to the presence of morphological factors such as hairs and trichomes, the thickness of plant tissues, and biochemical factors such as the presence of allelochemicals that adversely alter the insect behaviour, resulting in the insect moving away and selecting an alternate susceptible host plant (Painter 1951; Kogan 1982; Smith 1989).

Regarding the cocoa tree, when different genotypes are exposed to mirids in the form of fragments of twigs in the laboratory, the level of preference for these genotypes is expressed by the differences in the number of feeding lesions. In the field, this can be observed as differences in the level of dieback in the canopy, as a result of the damage caused by feeding. In the present study, the parental clones PA 150, T 79/501, NA 32, T 60/887 and UPA 402 and the hybrids progenies PA 150 x NA 32, T 79/501 x UPA 402 and P 7 x T 60/887 sustained low numbers of lesions compared to the other clones and hybrids. This indicates that these clones show some level of antixenosis.

Antixenosis was earlier identified to be one of the components of cocoa resistance to *S. singularis* (N'Guessan et al. 2004, 2008; Anikwe et al. 2009). Indeed, these researchers found the clone PA 150 to be antixenotic against *S. singularis*. On the other hand, the clone UF 676 which sustained high number of lesions in this study was found to sustain low number of lesions in the study carried out by Anikwe et al. (2009). This is probably because the clone UF 676 was more preferred by *S. singularis* in comparison with the other clones used in our study as opposed to the clones used by Anikwe et al. (2009). Nevertheless, the results obtained in this study confirmed in part those of Sounigo et al. (2003) who found the parental clones to be promising for mirid resistance, based on low field damages in clone trials.

Also, in our study the clones that exhibited little canker on the trunk and branches as a result of a low number of lesions (Sounigo et al. 2003) showed low numbers of lesions in the choice test in the laboratory. This indicates that the choice test, based on attractiveness of fragments of twigs, can be used to screen for resistance to cocoa mirids. Indeed, interesting results have been obtained by several scientists with regard to mirid resistance in cocoa (Nguyen-Ban 1994; N'Guessan et al. 2008; Anikwe et al. 2009). However in the field antixenosis can be reinforced by other factors. Studies by Lavabre (1977) indicated that clone UPA 134 was less attacked by mirids in the field compared to neighbours, as a result of the colour of the young leaves. Indeed, the colour of the leaves has an antixenotic effect on insects. Smith (1989) stated that antixenosis resistance in crop cultivars has been achieved by genetically altering the colour of plant foliage. Moreover, it has been shown that some cucurbit cultivars with silver-coloured leaves reflect more blue and ultraviolet wavelengths of light than normal cultivars and are resistant to aphids (Smith 1989). Similarly, it has been shown that the red leaf colour trait in cotton is a heritable character that causes antixenotic reactions in adult boll weevils, *Anthonomous grandis grandis* Boneman (Smith 1989).

Limited research has been conducted to investigate the cause of antixenosis in cocoa. The study conducted by Debost et al. (1988) showed that chemicals such as flavan-3-ols are involved in cocoa resistance to black pod disease. These chemicals may well be involved in cocoa resistance to mirids. Indeed, the work carried out by Cros et al. (1996) on young cocoa plants showed that two phenolic compounds, quercetin (flavonol 4) and kampferol (flavonol 7) may be involved in mirid resistance in cocoa. Nguyen-Ban (1994) stated that water content of the shoots may play an important role in the choice of food by cocoa mirids, but indicated that other factors including hairs on the shoots, colour of flushes and flavonol contents are involved in the food preference or non-preference by mirids. Nevertheless, further studies need to be conducted to elucidate the cause of antixenosis in cocoa.

Mirid resistance in the intra-population hybrids of the first cycle of the population breeding

In order to identify mirid resistant/tolerant genotypes among the intra-population hybrids of the first cycle of the population breeding, several hybrids were assessed in the field on the basis of cumulative mirid damage on trunks and branches. The hybrids were obtained from crosses between Upper-Amazons + Criollo and between Lower-Amazons + Trinitario. A rating scale of 0 to 5 was used, where 0 corresponds to no canker on the trunk and branches and 5 corresponds to all the trunk and branches covered with cankers.

The results showed that the level of attacks was relatively low on the individual trees of both populations (Upper-Amazons + Criollo and Lower-Amazons + Trinitario). Significant ($P<0.01$) differences were found between the intra Upper-Amazons + Criollo hybrids and between the intra Lower-Amazons + Trinitario hybrids regarding mirid damage (Tables 8 and 9). The results give valuable information on the genotypes selected for the second cycle.

Table 8. Behaviour of some intra-population Upper-Amazons + Criollo hybrids of the first cycle of the population breeding vis-à-vis cocoa mirids in the field, based on cumulative damage on trunk and branches

Hybrids	Mean rating score	Mean grouping
NA 32 x POR	1.92	a
IMC 67 x IFC 5	1.85	ab
IMC 67 x POR	1.84	ab
P 7 x POR	1.81	abc
SPEC 100-9 x PA 150	1.80	abc
EQX 94 x POR	1.80	abc
PA 150 x ICS 60	1.80	abc
P 10A x SCA 6	0.88	abc
T 60/887 x IMC 78	0.86	bc
UPA 413 x T 60/887	0.85	bc
T 79/501 x IMC 78	0.84	bc
T 60/887 x PA 150	0.84	bc
IMC 67 x IMC 78	0.83	bc
IMC 78 x T 60/887	0.79	c

Means followed by the same letters along the columns are not significantly different ($P>0.5$, Waller Duncan K ratio T test)

Table 9. Behaviour of some intra-population Lower-Amazons + Trinitario hybrids of the first cycle of the population breeding vis-à-vis cocoa mirids in the field, based on cumulative damage on trunk and branches

Hybrids	Mean rating score	Mean grouping
UF 221 x IFC 371	2.42	a
UF 676 x IFC 371	2.33	ab
ICS 46 x IFC 5	2.17	abc
IFC 5 x CC 10	2.13	abcd
IFC 5 x IFC 303	2.11	abcd
UF 211 x MAT 1-9	2.06	abcde
WA 40 x IFC 5	2.03	abcdef
IFC 1 x IFC 5	0.85	ghijkl
W 41 x IFC 303	0.83	hijkl
IFC 29 x N 38	0.79	ijkl
IFC 304 x IFC 303	0.76	jkl
IFC 6 x IFC 5	0.76	jkl
IFC 303 x IFC 1	0.68	kl
N 38 x IFC 5	0.66	l

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test)

Mirid resistance in the intra-population hybrids of the second cycle of the population breeding

This study aimed at obtaining information on the level of resistance to mirid of genotypes to be selected for the third cycle of the population breeding. The genotypes should be high-yielding and resistant to *Phytophthora* and mirids. The second cycle intra-populations hybrids of the Upper-Amazons and the Lower-Amazons + Trinitario were evaluated on the basis of damage on pods and cumulative mirid damage on trunks and branches as in the above test. The same rating scale was used, 0 corresponding to no damage on pods and 5 to all the pods showing mirid feeding lesions.

Regarding damage on pods, the results showed that the level of attacks was relatively low for both populations. Significant (P<0.01) differences were found between the hybrids in both the intra-population Upper-Amazons and the intra-population Lower-Amazons + Trinitario hybrids. The least and the most attacked hybrids are shown in Tables 10 and 11. In the Upper-Amazons hybrids populations, about 905 trees did not show any damage on pods, 178 trees sustained low damage and only 25 trees really showed substantial damage. Regarding the Lower-Amazons +Trinitario populations, 414 trees did not show any damage, 267 trees sustained low to medium level of damage, and 83 trees showed substantial damage. These results are interesting in the sense that there is a higher chance of selecting high-yielding, *Phytophthora*-resistant and mirid-tolerant trees for the third cycle, since many trees showed no or low level of damage.

With regard to the cumulative damage on trunk and branches, the results showed that damage level was also low on the individual trees of the two populations. Significant (P<0.01) differences were revealed among the Upper-Amazons hybrids and Lower-Amazons (+Trinitario) hybrids (Tables 12 and 13).

Overall, most of the hybrids that sustained no or low mirid damage on pods also sustained no or low mirid damage on trunk and branches. The results suggested that, with regard to mirid resistance/tolerance, many trees were promising and had potential to be selected for the third cycle of the population breeding.

Table 10. Mean rating score of mirid damage on pods of some intra-population Upper-Amazons hybrids of the second cycle of recurrent selection

Hybrids	Mean rating score	Mean grouping
(PA 150 x IMC 78) x (SCA 6 x T 60/887)	0.47	a
(P 7 x SCA 6) x (IMC 57 x PA 150)	0.44	ab
(T 60/887 x SCA 6) x (P 7 x T 60/887)	0.43	abc
ICS 1 x IFC 1	0.37	abcd
(MO 81 x SCA 6) x (PA 121 x PA 150)	0.33	abcde
(MO 81 x SCA 6) x (P 19/A x PA 150)	0	h
(IMC 57 x SCA 6) x (MO 98 x T 60/887)	0	h
(IMC 57 x SCA 6) x (MO 98 x T 60/887)	0	h
(UPA 413 x PA 150) x H 1258	0	h
(T 60/887 x PA 150) x (PA 13 x PA 150)	0	h
(UPA 409 x SCA 6) x (UPA 402)	0	h

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Table 11. Mean rating score of mirid damage on pods of some intra-population Lower-Amazons + Trinitario hybrids of the second cycle of recurrent selection

Hybrids	Mean rating score	Mean grouping
(IFC 1 x IFC 303) x (ICS 46 x IFC 303)	1.03	a
(GS 29 x VENC 4-11) x (WA 40 x IFC 371)	0.92	ab
(IFC 1 x IFC 303) x (WA 40 x IF C371)	0.90	abc
(IFC 303xN 38) x (IFC 303x GS 29)	0.80	abcd
(IFC 6 x IFC 5) x (W 41 x N 38)	0.77	abcde
(SNK 12 x IFC 371) x (W 41 x N 38)	0.20	fghi
(IFC 6 x MAT 1-9) x ICF 10	0.20	fghi
(IFC 304 x IFC 303) x (IFC 11 x MAT 1-9)	0.18	fghi
(IFC 303 x CC 10) x IFC 7	0.13	ghi
(IFC 303 x CC 10) x (IFC 11 x MAT 1-9)	0.11	hi
(IFC 304 x IFC 303) x (ACU 85 x MAT 1-9)	0.05	i

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test)

Table 12. Behaviour of some intra-population Upper-Amazons hybrids of the second cycle of the population breeding vis-à-vis cocoa mirids in the field, based on cumulative damage on trunk and branches

Hybrids	Mean rating score	Mean grouping
(AMAZ 15-15 x T 60/887) x (PA 13 x PA 150)	0.72	a
(AMAZ 15-15 x T 60/887) x (PA 121 x PA 150)	0.64	ab
(PA 150 x T 60/887) x (NA 32 x SC A6)	0.59	abc
ICS 1 x IFC 1	0.57	abcd
(P 7 x SCA 6) x (UPA 419 x UPA 419)	0.50	abcde
(T 79/501 x T 60/887) x (UPA 409 x UPA 409)	0.50	abcde
(MO 98 x SCA 6) x (UPA 608 x UPA 608)	0.49	abcde
11 (P 7 x SCA 6) x (IMC 57 x T 60/887)	0.47	abcdef
(AMAZ 15-15 x SCA 6) x (MO 98 x T 60/887)	0.12	efg
(PA 105 x IMC 78) x (SCA 6 x UPA 413)	0.11	efg
(MO 81 x SCA 6) x H 1258	0.11	efg
(SCA 6 x PA 150) x (PA 13 x IMC 67)	0.09	fg
(MO 81 x T 60/887) x (SCA 6 x UPA 413)	0.08	fg
(IFC 6 x MAT 1-9) x ICF 10	0.07	fg
(T 60/887 x PA 150) x (SCA 6 x T 60/887)	0.06	g
(NA 32 x SCA 6) x (PA 121 x PA 150)	0.06	g

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Table 13. Behaviour of some intra-population Lower-Amazons + Trinitario hybrids of the second cycle of the population breeding vis-à-vis cocoa mirids in the field, based on cumulative damage on trunk and branches

Hybrids	Mean rating score	Mean grouping
(W 41 x IFC 303) x (IFC 11 x MAT 1-9)	2.02	a
(IFC 304 x IFC 303) x (IFC 11 x MAT 1-9)	1.71	ab
(ICS 95 x MAT 1-9) x IRA 1	1.70	abc
(IFC 1 x IFC 303) x (ICS 46 x IFC 371)	1.69	abc
(IFC 6 x MAT 1-9) x ICF 10	1.64	abc
(ICS 95 x MAT 1-9) x IFC 18	1.62	abc
(ICS 84 x IFC 303) x DCG 1-1	1.62	abc
(GS 29 x N 38) x DCG 1-1	1.55	abc
(ICS 84 x IFC 303) x (ICS 46 x IFC 371)	0.90	bcd
(IFC 5 x IFC 15) x (IFC 8 x IFC 371)	0.89	bcd
(IFC 304 x IFC 303) x (IFC 29 x IFC 303)	0.85	bcd
(IFC 303 x CC 10) x IFC 7	0.84	bcd
(UF 676 x IFC 303) x IFC 18	0.84	bcd
(IFC 5 x IFC 15) x (ACU 85 x IFC 5)	0.76	bcd
(IFC 303 x N38) x (ICS 46 x IF C371)	0.75	cd
(IFC 303 x N 38) x (ICS 46 x IFC 303)	0.59	d

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Mirid resistance in the inter-population hybrids of the second cycle population breeding

Several hybrids of the second cycle of the population breeding obtained from crosses between Upper-Amazons genotypes and Lower-Amazons + Trinitario genotypes were evaluated on the basis of damage on pods in the field. The objectives were to assess the level of resistance/tolerance to mirids of high-yielding *Phytophthora*-resistant genotypes to be selected for confirmation trials and eventually varietal release.

The results showed that the level of mirid damage was low for the hybrids tested and no significant (P>0.05) differences were found between the genotypes. This suggested that hybrids for the confirmation trials could be selected just on the basis of yield and *Phytophthora* resistance since mirid damage was very low on all the hybrids. However, more series of tests may be necessary to confirm these results.

Mirid resistance in the regional hybrids trial

Cocoa hybrids of various origins (Ghana, Cameroun and Côte d'Ivoire) were evaluated for resistance/tolerance to mirids. The hybrids were planted in the Regional Variety Trial. Hybrids H1, H4, H6 and H9 from Côte d'Ivoire were used in the trial as controls. The hybrids were evaluated using the tolerance test based on *Lasiodiplodia* resistance. A fine needle was used to wound ten semi-hardened shoots of each hybrid, making two punctures on each internode to simulate lesions caused by mirids. The shoots were left to natural infestation by *Lasiodiplodia* for 7 weeks. After the 7-week-period, the shoots were cut off, brought to the laboratory and cut into fragments representing the internodes. The fragments were then split longitudinally to look for black stains on the inner side of the wounded areas. These marks were scored using a scale of 0 to 5 according to the size of the stain.

Table 14. Behaviour of selected regional hybrids to *Lasiodiplodia* infection after mechanical injury

Hybrids	Mean rating score	Mean grouping
GH 6	2.67	a
CI 1	2.51	ab
CI 3	2.36	bc
CI 2	2.35	bcd
GH 1	2.35	bcd
H 4	2.34	bcde
CI 4	2.30	bcdef
GH 4	2.28	bcdef
GH 7	2.27	bcdef
CA 2	2.14	cdef
GH 9	2.14	cdef
CA 3	2.13	cdef
GH 5	2.10	cdef
GH 8	2.10	cdef
GH 3	2.09	cdef
CA 4	2.08	cdef
CA 1	2.07	def
GH 2	2.05	ef
CI 5	2.03	f

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Significant (P<0.01) differences were found between the hybrids evaluated with regard to *Lasiodiplodia* infection. The hybrids CI 5 from Côte d'Ivoire, GH 2 from Ghana and CA 1 from Cameroun were found to be the least infected by *Lasiodiplodia* (Table 14). These hybrids were therefore promising for tolerance to mirids. On the other hand, the hybrids GH 6 from Ghana and CI 1 from Côte d'Ivoire were the most infected by *Lasiodiplodia* and therefore susceptible to mirids (Table 14). The promising hybrids are potential parents in the national breeding programme, especially materials from Ghana which are known for their tolerance to swollen shoot which is a problem in Côte d'Ivoire now.

Evaluation of 16 "international" clones

Twenty-four clones introduced in Côte d'Ivoire within the framework of the germplasm project were planted in a budwood garden on-station in Divo. Sixteen of these clones were evaluated using the tolerance test based on *Lasiodiplodia* resistance as above.

Significant (P<0.01) differences were found between the clones evaluated with regard to *Lasiodiplodia* infection. The clone VENC 4-4, with a score of 3.08 appeared to be more susceptible than the others, but differences were relatively low (Table 15). The clones PA 120, IFC 5, GU 255 and BE 10 were the least susceptible clones. These clones are potential parents in the national breeding programme.

Table 15. Behaviour of 16 international clones vis-à-vis *Lasiodiplodia* infection after mechanical injury

Clones	Mean rating score	Mean grouping
VENC 4-4	3.08	a
LCTEEN 46	2.78	b
IMC 47	2.58	bc
Mocorongo	2.57	bcd
T 85/799	2.54	bcd
NA 32	2.49	cde
PA 107	2.44	cdef
P 7	2.37	cdefg
PA 150	2.35	cdefg
T 79/501	2.29	defg
EQX 3360-3	2.22	efgh
ICS 1	2.19	fgh
BE 10	2.12	gh
GU 255V	2.09	gh
IFC 5	2.09	gh
PA 120	1.95	h

Means followed by the same letters along the columns are not significantly different (P>0.5, Waller Duncan K ratio T test).

Conclusion

Globally, the use of resistant varieties is an important component of integrated pest management and can easily be combined with other control methods. In this instance, the results obtained here are important. The existence of significant positive correlation between field damage and antixenosis in the laboratory indicated that antixenosis is a mechanism of mirid resistance in cocoa. In addition, results obtained with *Lasiodiplodia* resistance method showed that the size of the lesions caused by *Lasiodiplodia* after mechanical injury to cocoa twigs could be a good indicator of resistance to this pathogen and therefore an indicator of tolerance to mirid. More work will be conducted to refine the test and adapt it to seedlings for early screening of cocoa genotypes. Nevertheless, the hybrids and the clones that have shown some resistance/tolerance to mirids after the various screenings could be recommended for cultivation if they have good agronomic characteristics, or be integrated in a breeding programme to incorporate the resistance genes into suitable cultivars. Materials identified as promising for mirid resistance among the hybrids of the population breeding give an indication on the hybrids that could be selected for the next generation or for a confirmation trial, taking into account yield and *Phytophthora* resistance.

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Evaluation of mirid resistance at the Cocoa Research Institute of Nigeria

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Background

The brown cocoa mirid, *Sahlbergella singularis* Haglund (Hemiptera: Miridae), is the most harmful insect pest of the cocoa tree in Nigeria (Opeke 1992). Mirids feed by inserting their mouthparts into the plant and sucking the sap, simultaneously injecting salivary secretions into the tissue, which results in plasmolysis of the cells.

This cellular lysis results in necrosis, followed by the appearance of depressed oily spots known as lesions on the cocoa pods and branches (Mariau 1999). Lesions are circular on pods but oval and of somewhat greater size on stems (Wood and Lass 1989). Canker sores develop quickly from lesions due to invasion by cryptogamous parasites causing weakness. The combination of tissue necrosis and cryptogamic attack results in the wilting of the plant, leading to very low productivity (Mariau 1999). *Sahlbergella singularis* is the most prevalent insect pest in the cocoa belt of Nigeria and yield loss of about 30–70% has been attributed to mirid infestation and damage (Idowu 1989). Yield loss could be as high as 75% in cocoa farms attacked by the mirids and left unattended for a period of 3 years and above (Padi 1997).

The objectives of the cocoa improvement programme at the Cocoa Research Institute of Nigeria (CRIN) include breeding for higher yield, good quality and resistance to pests and diseases. With dieback incidence becoming significant in both budded and grafted cocoa, screening for mirid resistance gained prominence. The use of resistant varieties as an option for the control of this economically important insect pest species will be cheap and affordable to the resource-poor farmers, as well as ecologically sound. Therefore, under this project, the damage potential of *S. singularis* was evaluated on 44 cocoa genotypes for a period of 3 years (2004–06) and followed by testing for the mechanisms of resistance of these cocoa clones and hybrids.

Materials and methods

Field evaluation for damage caused by *S. singularis*

Screening of cocoa genotypes to damage by mirids was conducted on the CFC/CRIN/Bioversity Germplasm project plots. The material assessed included:

- 10 clones from the International Clone Trial plot with 3 plants per clone replicated in 3 blocks (10 x 3 x 3) – total of 90 plants
- 10 clones from the Local Clonal Trial Plot, 3 stands per clone in 3 blocks (10 x 3 x 3) – total of 90 plants; and
- 24 hybrids from the Hybrid Trial Plot by 3 stands by 4 blocks (24 x 3 x 4) – total of 288 plants.

The experiment layout followed the completely randomized blocks design. Table 1 lists all genotypes evaluated for mirid damage.

Table 1. List and key of cocoa genotypes (hybrids and clones) evaluated for resistance to *S. singularis* (N 38 was used as control)

Hybrids
1. T 65/7 x T 57/22
2. T 12/11 x N 38
3. T 65/7 x T 9/15
4. PA 150 x T 60/887
5. P 7 x T 60/887
6. P 7 x PA 150
7. T 65/7 x T 22/28
8. T 53/5 x N 38
9. T 65/7 x N 38
10. T 53/5 x T 12/11
11. T 65/35 x T 30/13
12. T 86/2 x T 9/15
13. T 9/15 x T 57/22
14. F3 Amazon
15. T 86/2 x T 22/28
16. T 82/27 x T 12/11
17. T 86/2 x T 16/17
18. T 65/7 x T 53/8
19. T 65/7 x T 101/15
20. T 86/2 x T 53/8
21. T 86/2 x T 65/35
22. T 101/15 x N 38
23. T 82/27 x T 16/17
24. T 86/2 x T 57/22

International clones
1. EET 59
2. VENC-4-4
3. PA 150
4. UF 676
5. AMAZ 15-15
6. BE 10
7. SPEC 54-1
8. T 85/799
9. Mocorongo
10. PA 107

Local clones
1. T 9/15
2. T 65/7
3. T 12/11
4. T 16/17
5. T 12/5
6. T 30/13
7. C 77
8. T 53/8
9. T 53/5
10. N 38

All selected plants were monitored at 2-weekly intervals for the following mirid damage parameters:

1. Presence of canker on trunks and main branches
2. Mirid incidence (lesions) on pods
3. Dieback of twigs.

Visual ratings of the trees were based on the methods used by Brun et al. (1997) and N'Guessan (2000) to assess damage due to canker on trunk and main branches, mirid damage (lesions) on pods and dieback of twigs (Table 2).

Three mechanisms of attractiveness and/or resistance were tested: antixenosis, antibiosis and tolerance. The clones and hybrids tested were the same as those listed in Table 1. Three additional genotypes later assessed for tolerance were IFC 5, Playa Alta and ICS 1.

Table 2. Key for visual rating scale for damage to *S. singularis* in the field

Plant part damaged	Damage index	Damage characteristics
Trunks and main branches	0	No canker found on trunks or main branches
	1	25% of the trunk and branch with cankers
	2	50% of the trunk and branch with cankers
	3	75% of the trunk and branch with cankers
	4	Almost all the entire trunk and branches cankered
Pods and cherelles	0	No lesions found on pods
	1	25% of pods and cherelles with presence of lesions
	2	50% of pods and cherelles with presence of lesions
	3	75% of pods and cherelles with presence of lesions
	4	Almost all the pods showing symptoms of lesions
Dieback of twigs and leaves	0	No dieback
	1	25% of leaves and twigs showing dieback
	2	50% of leaves and twigs showing dieback
	3	75% of leaves and twigs showing dieback
	4	Almost all the leaves and twigs showing dieback

Antixenosis

This study was carried out to assess the ability of selected cocoa lines to attract or repel mirid attack in the laboratory. The genotypes tested for antixenosis are listed in Table 1. One-day-old adult mirids were collected carefully very early in the day using a haemolysis tube and a camel hairbrush from the insect culture in the laboratory. After collection, the insects were allowed to rest and were left unfed overnight to encourage their feeding on the plant materials to be provided during the experiment the following day.

Green twigs of expanded young flushes (about 2 weeks old) were used for the test. These twigs were cut into 50-mm-long pieces. Twigs of the same diameter were used and laid out in triangles fitting within a Petri dish (Plate 1). The twigs were stapled together and each side of the triangle consisted of a different cocoa genotype. Each genotype was replicated 20 times in a completely randomized design. Five replicates of each of the series were carried out on the same day and repeated four times until all the 20 replicates were tested. The Petri dishes were checked 24 hours later for mirid feeding punctures, which were easily recognized through the dark patches left at the feeding sites on the cut twigs from the different genotypes. An analysis of variance (ANOVA) was carried out to class genotypes in increasing or decreasing order of attractiveness.

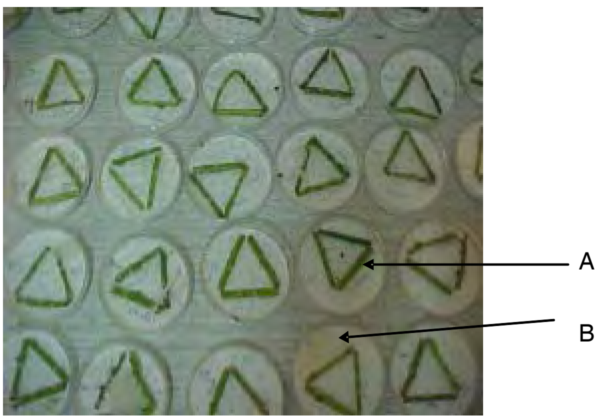


Plate 1. Laboratory microtest for antixenosis of *S. singularis* on cocoa genotypes.
A = 50-mm twig of cocoa genotype
B = Petri dish (9 cm diameter) lined with filter paper

Antibiosis

The objective of this study was to assess the survival and development of cocoa mirids on selected cocoa genotypes in the field. The material evaluated included 25 cocoa genotypes (6 local clones, 7 international clones and 12 hybrids). A no-choice test was used. The design was a completely randomized blocks design. Second-instar nymphs were collected from the stock culture of mirids reared in the entomology laboratory. A muslin mesh sleeve cage measuring 170 cm by 30 cm was used to confine two second-instar nymphs on a mature green pod in the field. Both ends of the cage were securely tied to prevent the insects from escaping. Grease was applied at both ends of the branch holding the pod to prevent ants from attacking the mirids in the sleeve cage. There were five replicates per genotype, giving a total of ten mirids being assessed per genotype. The mortality of the nymphs was recorded every 2 days to assess mirid survival on the different genotypes.

Tolerance

The study was conducted on 25 genotypes with the objective of identifying cocoa genotypes capable of withstanding or recovering from mirid damage. The experiment was based on the assessment of the level of dieback of twigs and recovery from damage in response to mirid feeding punctures. A completely randomized blocks design was used. A sleeve cage (170 cm height x 30 cm diameter) made with muslin cloth was used to confine one-day-old adult mirids, which must have been starved overnight, on a single twig for 48 hours. Five sleeves were placed on one tree and this constituted one replicate. This experimental set-up was repeated on one plant per block (three blocks in all), giving a total of 15 mirids per genotype. The insects were then removed after 48 hours but the sleeves were left in the field to prevent other insect pests' damage to the twigs. The physiological reaction of the shoot was observed weekly for 3 months. Each genotype was also wounded mechanically with the aid of a pin (10 punctures per plant twig on each internode, n=10) to serve as control. The following data were recorded:

- The physiological reaction of the twigs was assessed once a week during the first month and monthly thereafter for three months. A 5-point scale was used with 0 = completely healthy twigs to 4 = dead twigs.
- The length of progression of dieback of twigs of each genotype was measured with a measuring tape.

All data obtained were subjected to ANOVA and means separated using the Tukeys' Honestly Significant difference at 5% probability.

Results and discussion

Figures 1 and 2 show the damage profile of *S. singularis* in terms of lesions created on pods of selected genotypes in Ibadan. Among the hybrid genotypes evaluated for lesions, dieback of twigs, and canker on trunks, those showing least symptoms of mirid damage within the years under study were:

T 86/2 x T 16/17	T 65/35 x T 30/13
T 101/15 x N 38	T 86/2 x T 65/35
T 65/7 x T 22/28	T 65/7 x T 53/8
P 7 x T 60/887	T 65/7 x T 101/15
T 82/27 x T 12/11	T 53/5 x T 12/11

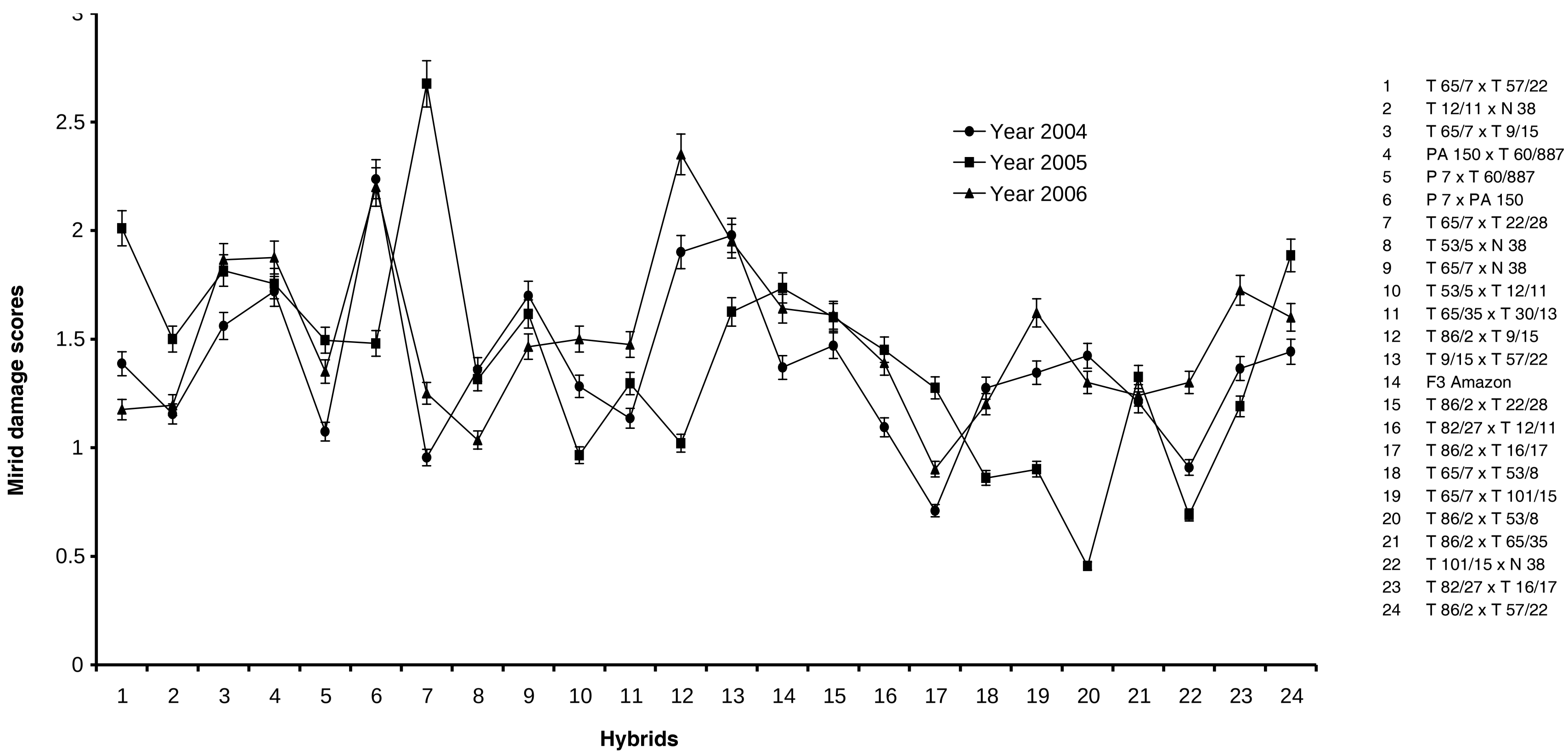


Figure 1. Mean scores ± SE (n=12) of mirid lesions on hybrid genotypes (cocoa pods) at CRIN Headquarters, Ibadan (2004-06).

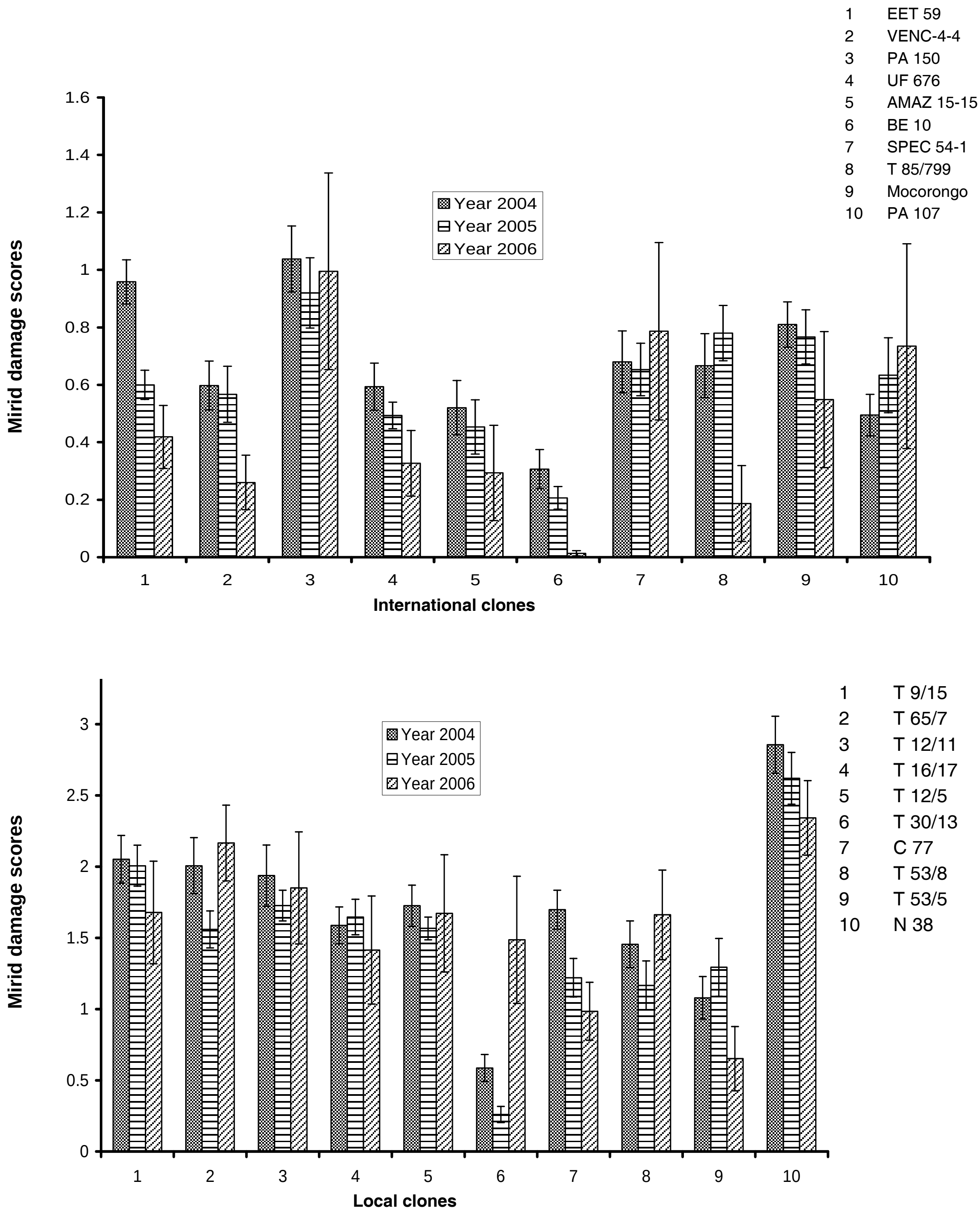


Figure 2. Mean scores of mirid lesions on international and local cocoa genotypes at CRIN Headquarters, Ibadan (2004–06). Values shown are mean ± SE (n=9).

Figures 3 and 4 show the mean scores of mirid damage in terms of twig dieback while Figures 5 and 6 show the damage profile of *S. singularis* in terms of cankers on trunks and branches of selected genotypes. EET 59, VENC 4-4, UF 676, AMAZ 15-15, BE 10, Mocorongo and PA 107 were among the outstanding genotypes in the field in respect of all the mirid damage symptoms evaluated (Figures 1-6). The local clones generally had high scores for mirid lesions, twig dieback and cankers, thereby suggesting susceptibility of such genotypes to mirid infestation. However, T30/13 was the only local clone that had least damage scores to mirid lesions, dieback of twigs and cumulative cankers and was found to be consistent over the 3-year period.

N 38, a local genotype used as control, had very high mean scores for damage parameters measured. In 2004, 2005 and 2006, N 38 had mean values of 2.85, 2.52, and 2.34 for lesions, respectively; 1.94, 2.08, 1.54 for dieback, respectively; and 2.97, 3.13, 2.21 for canker, respectively. These were very high scores when compared to the other genotypes.

A similar work was carried out by N'Guessan et al. (2006) who screened 500 cocoa clones in Côte d'Ivoire, out of which VENC 4-4, BE 10 and AMAZ 15-15 were identified as being among the resistant genotypes. Although the genotypes screened by N'Guessan are not all present in Nigeria, the result from this work appears to be in consonance as VENC 4-4, BE 10 and AMAZ 15-15 were found to be equally resistant to mirid in the field in Nigeria.

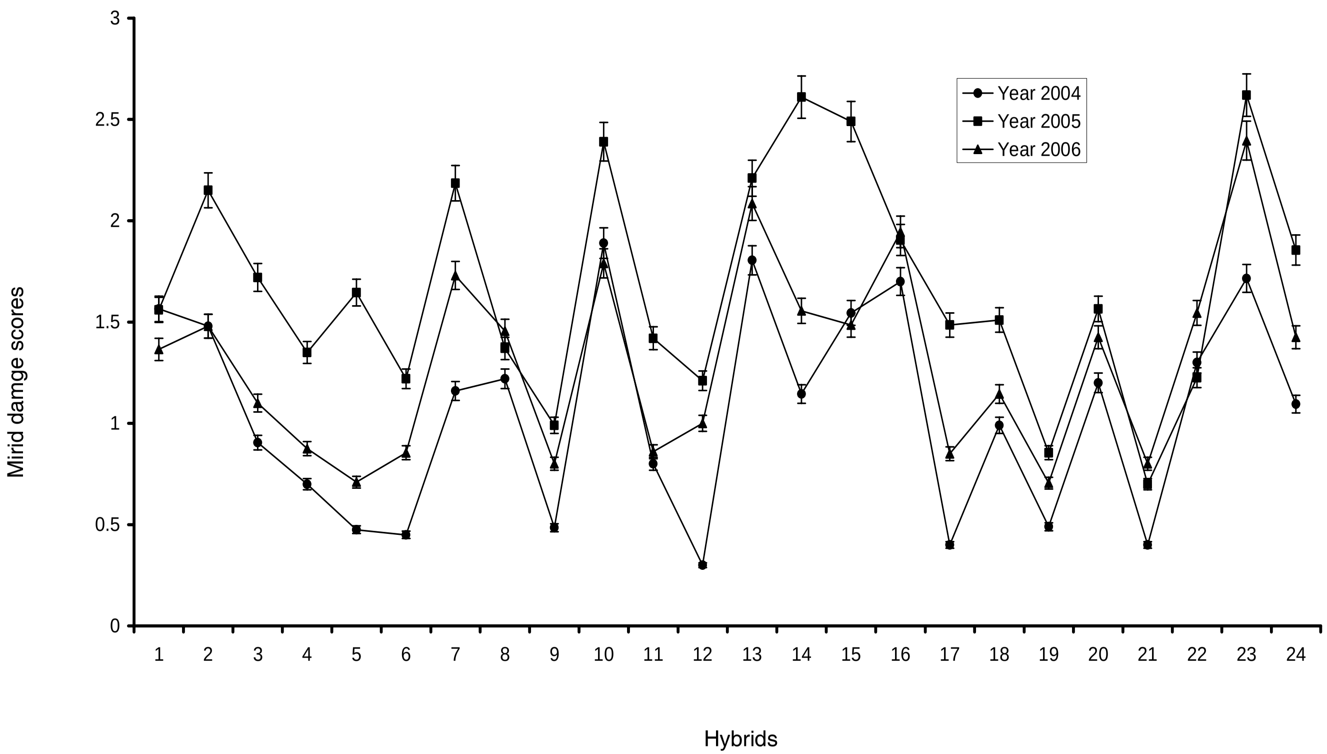
Figure 7 shows the result of antixenosis on 24 hybrid genotypes while N 38 served as control. Hybrids with a mean number of feeding points lower than 2 were adjudged to be least attractive to the brown cocoa mirid. These were:

T 65/7 x T 57/22	F3 Amazon
T 65/7 x T 9/15	T 86/2 x T 16/17
P 7 x PA 150	T 65/7 x T 53/8
T 53/5 x N 38	T 86/2 x T 65/35
T 53/5 x T 12/11	T 82/27 x T 16/17
T 65/35 x T 30/13	

Antixenosis results showed hybrid T 86/2 x T 16/17 to be the least attractive with a mean feeding point of 0.36±0.36 whereas the most attractive genotype was (T 86/2 x T 57/22) with a mean feeding point of 4.79±1.41.

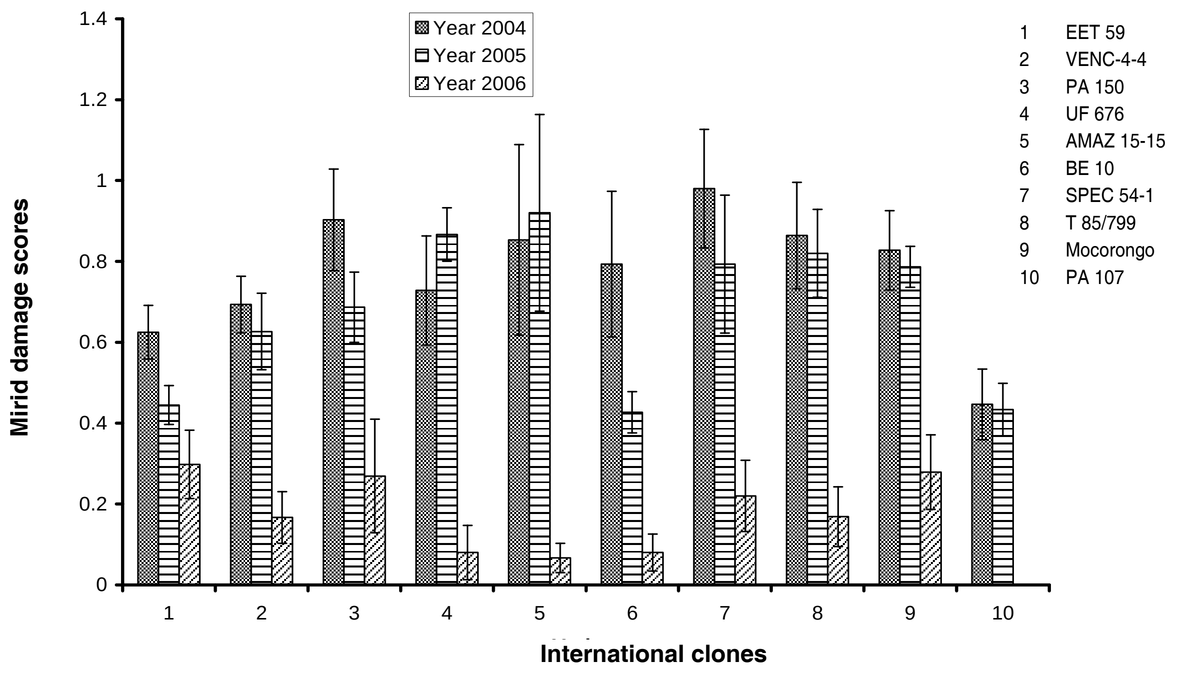
The result of antixenosis on local clones is presented in Figure 8. Among the ten selected genotypes tested, T 9/15 had no mirid bite on any of its twigs 24 hours after mirid introduction in the Petri dishes. Genotypes T 12/5, T 30/13, C 77, T 53/8 and T 53/5 were equally least attractive to mirid with mean feeding points of 0.67±0.58, 1.17±0.66, 1.25±1.08, 0.08±0.10 and 0.92±0.75, respectively.

Figure 9 shows the result of antixenosis on ten selected international clones in the laboratory. Clones EET 59, PA 150, UF 676, AMAZ 15-15, BE 10, SPEC 54-1 and PA 107 were least attractive to mirids in the laboratory with a range of feeding points of 0.17 to 1.08. SPEC 54-1 had the least mean of 0.17±0.11 whereas Mocorongo was the most attractive genotype with mean feeding points of 3.67±1.35.



1 T 65/7 x T 57/22
2 T 12/11 x N 38
3 T 65/7 x T 9/15
4 PA 150 x T 60/887
5 P 7 x T 60/887
6 P 7 x PA 150
7 T 65/7 x T 22/28
8 T 53/5 x N 38
9 T 65/7 x N 38
10 T 53/5 x T 12/11
11 T 65/35 x T 30/13
12 T 86/2 x T 9/15
13 T 9/15 x T 57/22
14 F3 Amazon
15 T 86/2 x T 22/28
16 T 82/27 x T 12/11
17 T 86/2 x T 16/17
18 T 65/7 x T 53/8
19 T 65/7 x T 101/15
20 T 86/2 x T 53/8
21 T 86/2 x T 65/35
22 T 101/15 x N 38
23 T 82/27 x T 16/17
24 T 86/2 x T 57/22

Figure 3. Mean scores of twig dieback caused by *S. singularis* on hybrid genotypes at CRIN Headquarters, Ibadan (2004-06). Values shown are means ± SE (n=12).



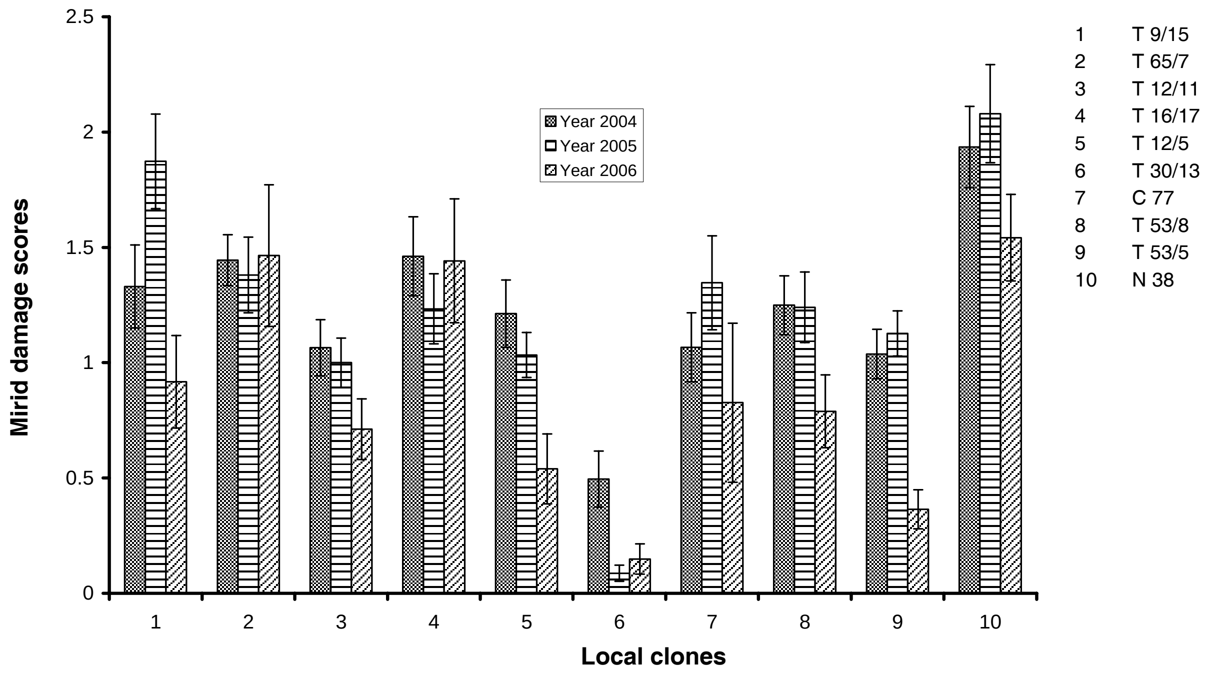


Figure 4. Mean scores of twig dieback caused by *S. singularis* on international and local cocoa genotypes at CRIN Headquarters, Ibadan (2004–06). Values shown are means ± SE (n=9).

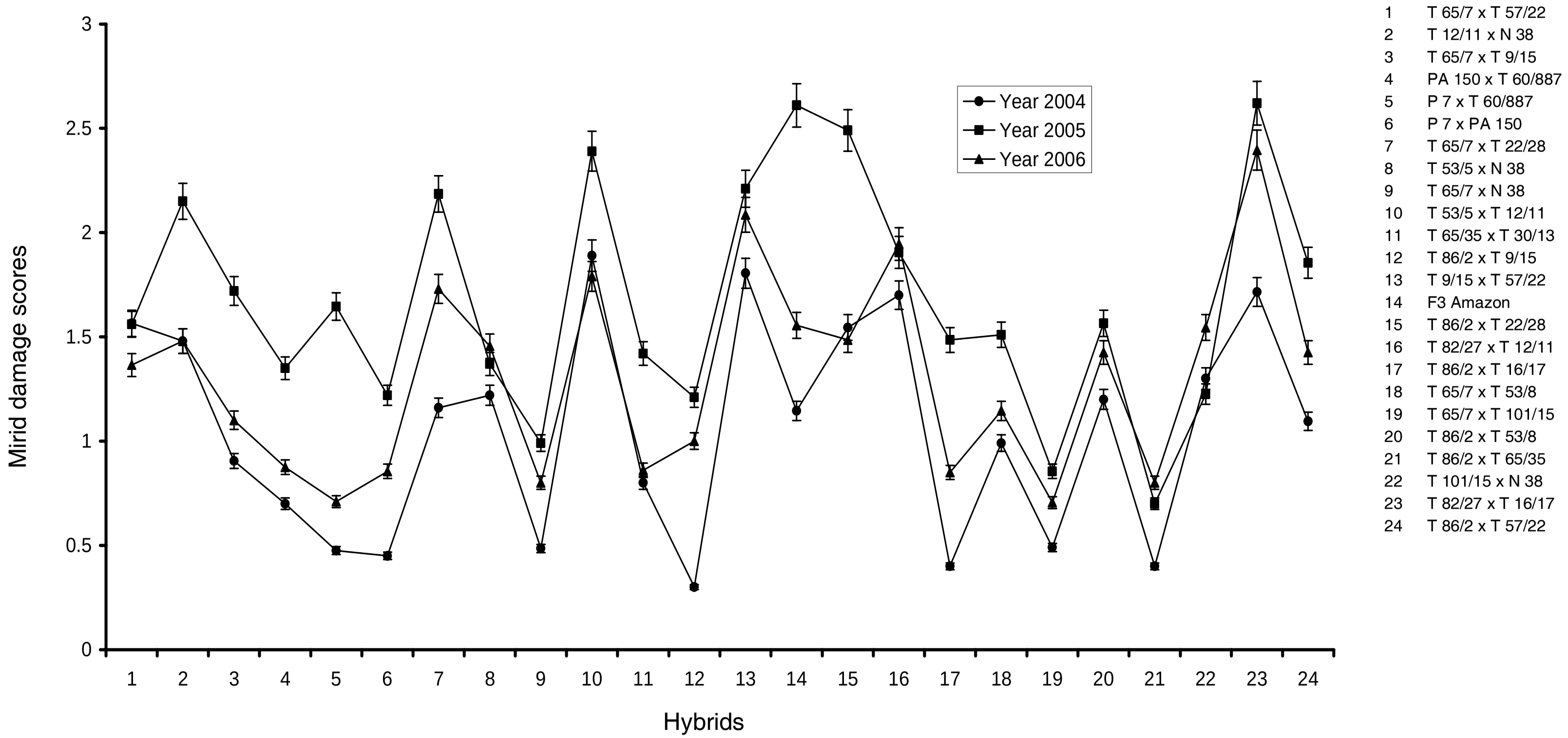


Figure 5. Mean scores of cankered trunks and branches caused by *S. singularis* on hybrid genotypes at CRIN Headquarters, Ibadan (2004-06). Values shown are means ± SE (n=12).

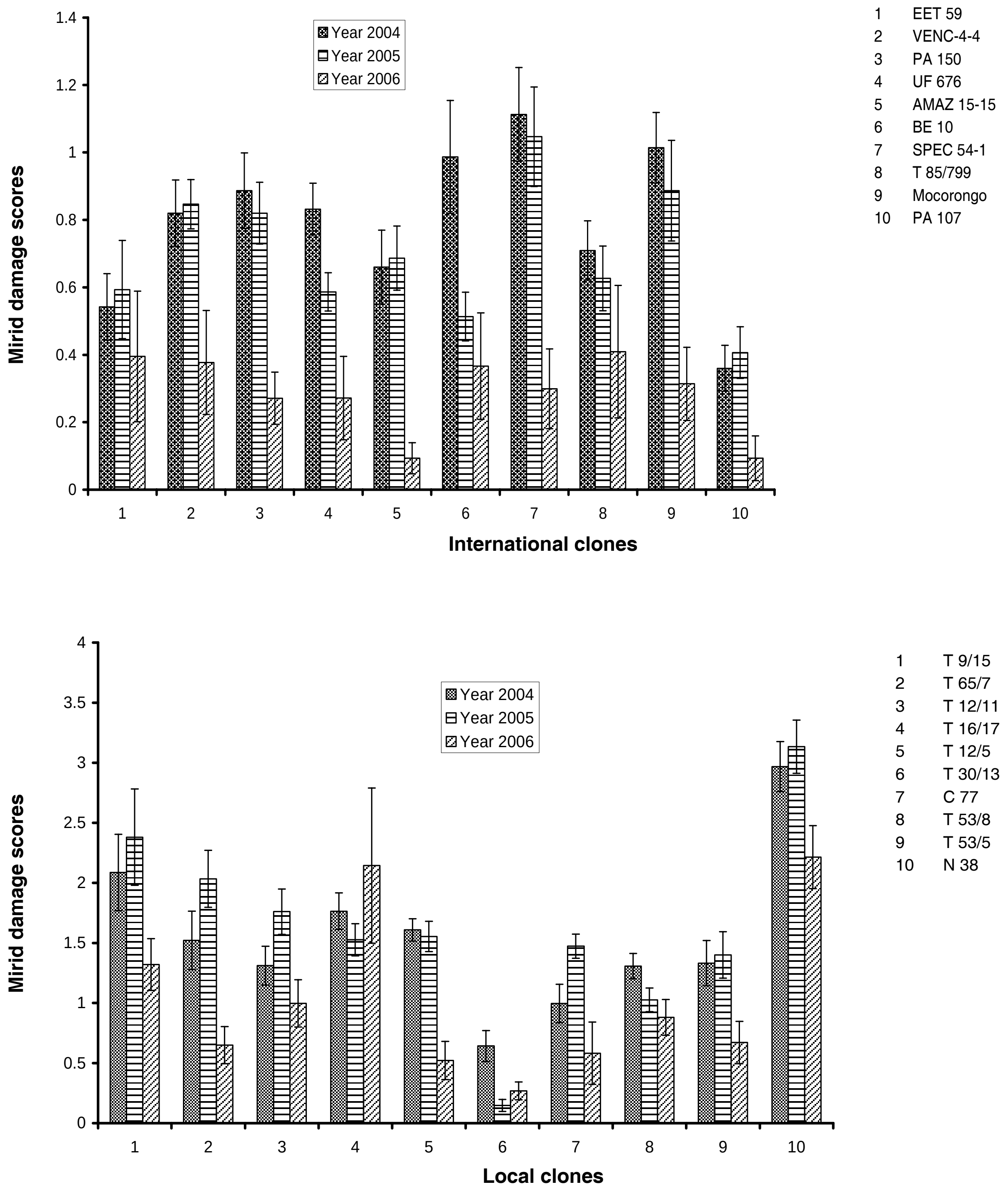


Figure 6. Mean scores of cankered trunks and branches caused by *S. singularis* on international and local cocoa genotypes at CRIN Headquarters, Ibadan (2004–06). Values shown are means ± SE (n=9).

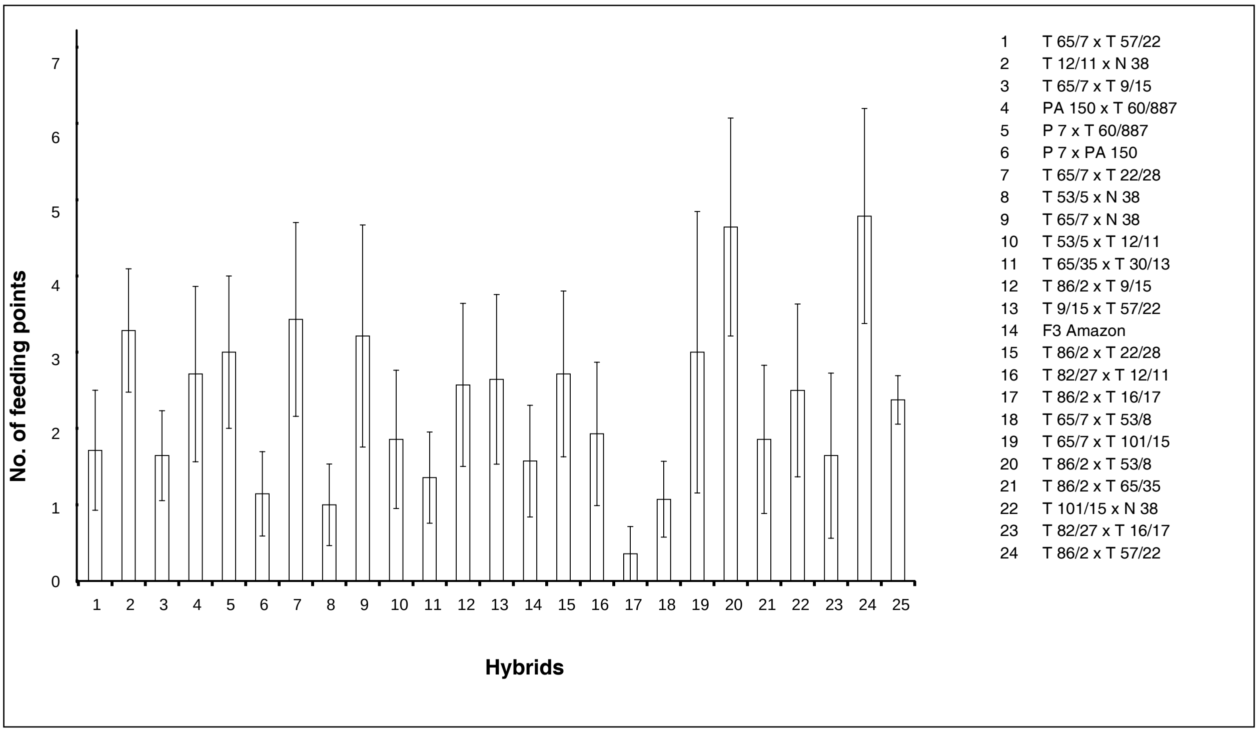


Figure 7. Laboratory microtest of antixenosis of *S. singularis* on cocoa hybrid genotype twigs. Values shown are means ± SE (n=20). Genotype 25 is the N 38 control variety.

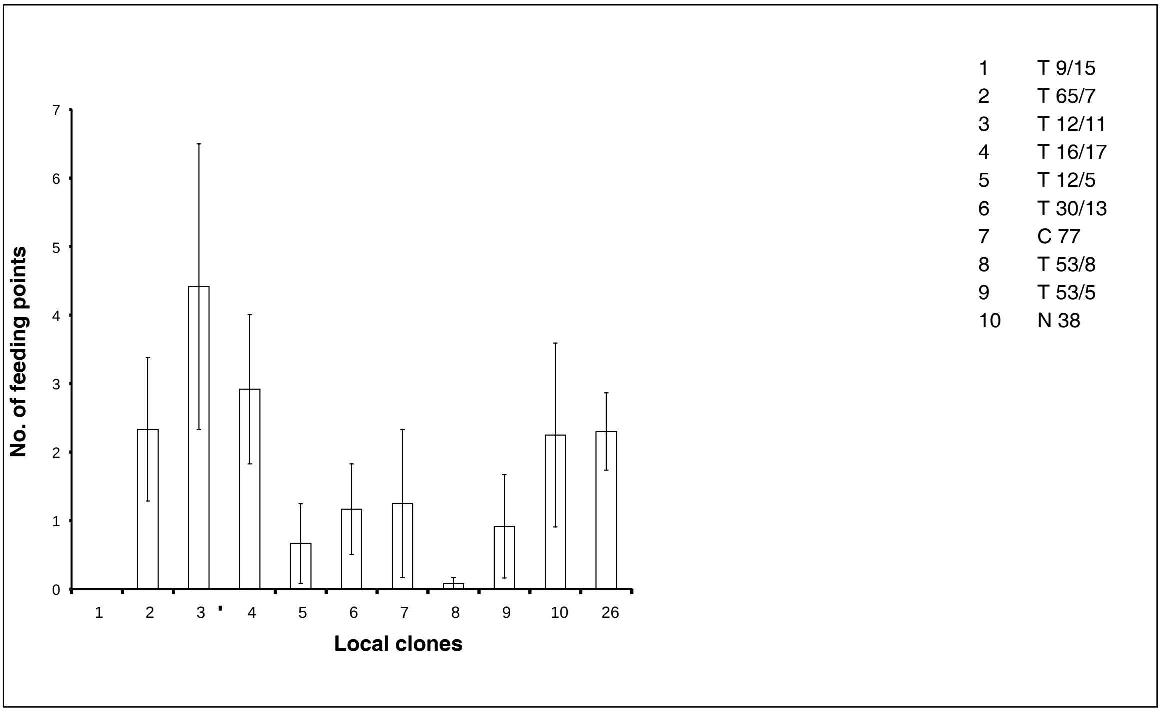


Figure 8. Laboratory microtest of antixenosis of *S. singularis* on local cocoa genotype twigs. Values shown are means ± SE (n=20). Genotype 26 is the N 38 control variety.

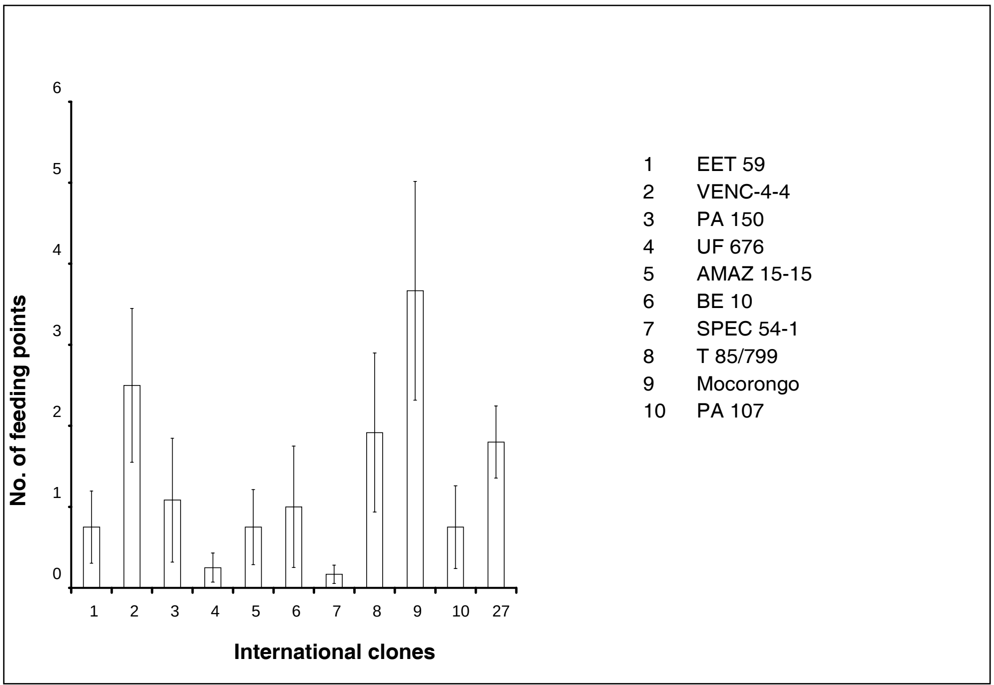


Figure 9. Laboratory microtest of antixenosis of *S. singularis* on international cocoa genotype twigs. Values shown are means ± SE (n=20). Genotype 27 is the N 38 control variety.

Twenty-five genotypes were tested for antibiosis (Table 3). EET 59 was the best genotype in terms of antibiosis with only 28.7% of mirid survival from first instar to adult. There were significant differences among the genotypes with respect to mirid survival on cocoa twigs using the Tukey's Studentized Range Test (HSD) at P=0.05. There was a relatively large overlap in the groups of significance using the Tukey's Studentized Range Test (HSD) at P=0.05.

Twenty-eight genotypes were assessed for tolerance, i.e. their ability to withstand attack by the brown cocoa mirid, *S. singularis*. Table 4 shows the parameters tested in terms of tolerance, which includes recovery ability of twigs and progression of dieback on twigs. In this study, tolerance to cocoa mirid was based on assessment of the cocoa plant reaction to attacks, starting 48 hours after the introduction of mirids to selected cocoa twigs. Twigs from genotypes N 38, T 30/13, T 53/8 and T 65/7 x T9/15 could hardly recover from mirid attacks (Table 4).

Dieback progression from pin punctures and mirid stylets showed that PA 107, SPEC 54-1 and AMAZ 15-15 had very low dieback progression. For instance, PA 107 had mean lengths of dieback of 1.2 mm from pin punctures and 3.2 mm from mirid attack (Table 4) and is therefore adjudged the best genotype to slow down dieback. Genotype T 65/7 x T 53/8 had the worst result in terms of tolerance with mean lengths of 9.6 mm and 8.1 mm from mirid attack and pin punctures, respectively. UF 676 which performed very well in terms of antixenosis and antibiosis showed a relatively moderate result in terms of tolerance. This was equally true for genotypes C 77, PA 150 and F3 Amazon.

Similar work carried out by N'Guessan (2006) in Côte d'Ivoire showed genotypes T 79/501, UPA 134, ICS 60, UPA 409, PA 150, IMC 57, IFC 14, N 38, R 15, IFC 6, IFC 15 and IFC 5 as well as international clones such as Playa Alta 2, PA 107 and EET 59 among others as being antixenotic. The results obtained in terms of antixenosis in this present study is partly in consonance with those obtained by N'Guessan (2006) in which EET 59, PA 150, UF 676, AMAZ 15-15, BE 10, SPEC 54 and PA 107 (international clones) and some hybrid crosses showed lower number of lesions. However, not all the genotypes tested in Côte d'Ivoire were assessed in this study in Nigeria. The result of antixenosis also agrees with Sounigo et al. (1994) who found T 79/501 and PA 150 as tolerant based on cumulative damage observed in the field.

Genotypes BE 10, AMAZ 15-15, SPEC 54-1, UF 676, P 7 x PA 150 and PA 107 which did very well in antixenosis were also found to have low survival of mirid nymphs. Although the causes of antibiosis have not been investigated in this study, previous works on other crops have attributed antibiosis to either presence of toxins or growth inhibitors in the plant (Smith 1989).

The ability of the cocoa twigs to recover from mirid attack was an important parameter measured for tolerance. Genotypes ICS 1, EET 59, BE 10, AMAZ 15-15, SPEC 54-1 and PA 107 had good recovery rates and at the same time, had low levels of dieback. Also some genotypes with high number of mirid lesions, low dieback and good rate of recovery such as UF 676, C 77, PA 150 and F3 Amazon were described as tolerant. The implication of this finding is that these genotypes will be able to maintain more flower cushions and yield better in the field than genotypes with low tolerance. The result of this study is in consonance with that of Babin et al. (2004) who found that UF 676 and IMC 60 showed the best ability to contain the degree of damage, while Playa Alta 2, ICS 1 and UF 676 were the best clones in terms of ability to recover from mirid damage in Cameroon.

Table 3. Survival of mirid nymphs on cocoa twigs of selected cocoa genotypes

Accessions	Survival rate	Mean grouping[1]
N 38	100.00	a
T 65/7 x T 53/8	100.00	a
T 9/15	100.00	a
T 53/5 x N 38	98.90	a
T 30/13	98.90	a
T 65/35 x T 30/15	98.90	a
T 12/5	97.80	a
T 53/8	97.80	a
T 82/27 x T 16/17	97.80	a
T 86/2 x T 65/35	96.70	a
T 86/2 x T 16/17	95.60	a
T 65/7 x T 9/15	94.50	a
T 53/5 x T 12/11	94.30	a
F3 Amazon	92.30	a
T 65/7 x T 57/22	92.30	a
C 77	92.30	a
T 82/27 x T 12/11	91.20	a
PA 150	78.90	ab
PA 107	67.80	bc
P 7 x PA 150	61.20	bc
UF 676	56.80	c
SPEC 54-1	54.50	c
AMAZ 15-15	54.50	c
BE 10	52.20	c
EET 59	28.70	d

[1] Tukey's Studentized Range (HSD) Test. Means followed by the same letter in the same column are not significantly different (P>0.05)

Table 4. Mirid feeding lesions, recovery from fresh mirid feeding lesions and progression of dieback from mirid lesions and from mechanical wounding

Lines	Feeding lesions		Recovery rate		Dieback progression (mm)			
					Mirid		Pin	
N 38	12.2	a[1]	3.6	a	8.4	abcd	7.3	abc
T 65/7 x T 53/8	9.9	abcd	2.8	abcd	9.6	a	8.1	a
T 9/15	10.3	abc	3.1	abc	9.0	ab	7.3	abc
T 53/5 x N 38	10.2	abc	3.2	abc	7.8	abcde	7.7	ab
T 30/13	10.7	abc	3.4	ab	6.7	bcdef	6.0	abcde
T 65/35 x T 30/15	9.9	abcd	2.9	abc	7.8	abcde	6.9	abcd
T 12/5	10.5	abc	2.5	abcdef	8.6	abcd	7.3	abc
T 53/8	11.0	ab	3.5	a	8.7	abc	7.3	abc
T 82/27 x T 16/17	10.5	abc	3.2	abc	9.0	ab	8.0	a
T 86/2 x T 65/35	10.0	abc	3.2	abc	8.8	ab	7.5	ab
T 86/2 x T 16/17	10.1	abc	3.0	abc	9.1	ab	8.2	a
T 65/7 x T 9/15	9.6	abcd	3.4	ab	8.8	ab	7.8	ab
T 53/5 x T 12/11	9.8	abcd	2.5	abcdef	8.0	abcde	7.1	abcd
F3 Amazon	9.8	abcd	2.6	abcde	5.9	defgh	5.4	bcdef
T 65/7 x T 57/22	10.0	abc	3.0	abc	8.9	ab	8.1	a
C 77	9.6	abcd	2.6	abcde	4.4	fghi	3.6	efgh
T 82/27 x T 12/11	9.0	bcd	3.0	abc	7.4	abcde	6.9	abcd
PA 150	8.0	bcde	2.2	cdefg	6.0	cdefg	3.7	efg
PA 107	6.8	de	1.5	efg	3.2	hi	1.2	h
P 7 x PA 150	7.8	cde	2.3	bcdefg	5.6	efgh	4.1	ef
UF 676	5.8	ef	2.3	bcdefg	4.3	fghi	3.7	efg
SPEC 54-4	5.5	ef	1.4	fg	2.8	i	3.0	fgh
AMAZ 15-15	5.5	ef	1.7	defg	2.8	i	1.6	gh
BE 10	5.3	ef	1.4	fg	3.4	ghi	4.7	def
EET 59	2.7	f	1.4	fg	5.6	efgh	5.0	cdef
IFC 5	3.3	f	1.6	efg	3.7	ghi	4.1	ef
Playa Alta 2	5.2	ef	2.3	bcdefg	4.4	fghi	3.9	efg
ICS 1	6.8	de	1.2	g	5.6	efgh	4.8	def

[1] Tukey's Studentized Range (HSD) Test. Means followed by the same letter in the same column are not significantly different ($P>0.05$)

Seven genotypes selected as being resistant to the brown cocoa mirid from this study include EET 59, BE 10, AMAZ 15 and UF 676, PA 107, SPEC 54-1 and P 7 x PA 150. Table 5 shows the basis for their selection.

Table 5. Basis for selection of identified resistant genotypes of cocoa

Genotypes	Basis of selection	Rating	Remarks
EET 59	Antixenosis	0.75	Good
	Antibiosis	28.7%	Best genotype in terms of antibiosis
	Recovery	1.4	High recovery rate
BE 10	Antixenosis	1.00	Good
	Antibiosis	52.2%	Moderately resistant
	Recovery	1.4	High recovery rate
AMAZ 15-15	Antixenosis	0.75	Good
	Antibiosis	54.5%	Moderately resistant
	Recovery	1.7	High recovery rate
UF 676	Antixenosis	0.25	Good
	Antibiosis	56.8%	Moderately resistant
	Recovery	2.3	High recovery rate
SPEC 54-1	Antixenosis	0.16	Best genotype for non-preference
	Antibiosis	54.5%	Moderately resistant
	Recovery	1.4	High recovery rate
PA 107	Antixenosis	0.75	Good
	Antibiosis	67.8%	Moderately resistant
	Recovery	1.7	High recovery rate
P 7 x PA 150	Antixenosis	1.14	Good
	Antibiosis	61.2%	Moderately resistant
	Recovery	2.3	High recovery rate

Conclusions and recommendations

There is no doubt this work has brought out some cocoa genotypes showing consistency in all mechanisms of resistance tested. Host plant resistance is the inherited ability of a plant species to ward off or resist attack by pests or to be able to tolerate damage caused by pests. Resistant varieties are one of the important components of pest management and can easily be combined with other control methods.

It is also expected that in due course, these resistant genotypes to *S. singularis* will be made available to cocoa farmers in the 14 cocoa-producing states through the establishment of seed gardens in the framework of the ongoing Cocoa Seed Garden Project (SGP). Once these genotypes are established within the farmers' domain, planting materials become readily accessible to the farmers. This is geared towards having improved cocoa varieties that are highly precocious, high-yielding and pest-resistant. Furthermore, breeding for resistance should be carried out continuously to select new varieties to replace older ones.

It is important to further investigate the basis of resistance of resistant cocoa genotypes while testing for mechanisms of resistance should be continued.

Impact of study on farmers, national research objectives and scientists

Part of the objectives of the cocoa improvement programme at the Cocoa Research Institute of Nigeria which included breeding for resistance to pests and diseases has been achieved. The results of this study have therefore led to the identification of resistant cocoa genotypes to *S. singularis* that could be a component part in the development of an Integrated Pest Management (IPM) strategy for the brown cocoa mirid in Nigeria. When these resistant cocoa genotypes will be available to farmers, the cost of inputs required for the control of this obnoxious insect pest through pesticide usage will be greatly minimized, with attendant increase in yield and quality of cocoa produced.

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Germplasm enhancement for resistance to black pod and witches' broom in Trinidad

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Abstract

In the first-cycle progeny of the germplasm enhancement programme for black pod (BP) resistance, observations were carried out 25 times on 866 trees in Field 14 (located at La Reunion Estate, Centeno) and on 768 trees in Field 7 (located at the International Cocoa Genebank, Trinidad (ICG,T)) for BP and witches' broom (WB) resistance, vigour and precocity. Screening for BP resistance was carried out on 766 genotypes using the detached pod test and confirmatory testing was done for 367 of these. Bean number and bean weight were recorded for 762 genotypes with 1–10 pods. Data were collected on 5-10 pods for 451 of these genotypes.

As part of the second-cycle progeny 1017 seedlings generated from 24 crosses using first-cycle progeny trees as parents were established in the greenhouse. Early screening for resistance to BP was successfully completed on 1013 seedlings and 411 (41%) were found to be highly resistant. Selection of plants with high levels of resistance to BP was completed and these resistant plants were established in Field 7.

Evaluation for WB disease resistance was carried out on 211 clones via manual spray inoculation method as part of the WB screening programme. Clones were assessed for resistance based on the number of inoculated shoots developing symptoms after inoculation, expressed as a percentage of the overall number of shoots inoculated per clone.

Following mass screening, 94 promising clones were re-tested for confirmation of WB resistance using a more sensitive agar-droplet screening method. Plants were evaluated based on symptom severity (incubation period and broom-base diameter) and results were analysed by analysis of variance (ANOVA) using the general linear model.

In the germplasm enhancement programme for WB resistance, 63 crosses were completed over 3 years, including 10 reciprocal crosses from year 1 following the incomplete diallel Kempthorne and Curnow model. Screening for WB resistance was carried out for 5300 seedlings from these crosses and 1480 seedlings showed moderate to high resistance based on agar-droplet inoculation. Sixty percent of the seedlings selected for WB resistance were also screened for BP resistance using the leaf disc method. Out of 134 seedlings from 28 year-1-crosses which were planted in the field, 110 were resistant to both WB and BP. Fifty-five WB-susceptible seedlings, together with parental grafts, were planted together with disease-resistant material for future field comparisons.

APPENDICES

Appendix I. Conclusions and recommendations from the Workshop

Session 1. Salient features of the farm survey and of the on-farm trials

Session 1.1. Salient features of the farm survey and collecting of interesting trees

Topics

- Knowledge of farmers on their planting materials
- Number of interesting trees identified
- Number of trees established in Farm Selection Observation Plots (FSOPs)
- Evaluation of farm selections for disease resistance
- Use of selected trees in on-farm trial plots.

Conclusions and recommendations

- Often mixtures of traditional and improved varieties are found in farmers' fields.
- The surveys revealed that many farmers are ageing as well as farmers' plantations.
- Farmers are able to identify materials by characteristics (yield, resistance and pod/bean characteristics).
- Some preference for "traditional"/own material with regard to black pod incidence was observed.
- Many farmers are willing to accept (and some to pay for) improved types looking for yield, disease/pest resistance and pod/bean characteristics.
- It is essential for breeders to interact more with farmers to understand farmers' needs and growing conditions.
- Farmers' knowledge and material can be useful for research (e.g. farmer selections for black pod correlated with leaf disk screening).
- Knowledge of farmers is based mostly on observation of individual trees, though some have the ability to identify varieties based on pod characteristics.
- Due to low heritability of traits from single tree selections, further evaluation is essential to assess true genetic potential.
- Farmer field conditions are different from research station (environment and inputs). This is to be taken into account when assessing farmers' materials.
- The potential of improved materials on-farm is often not being realized due to low inputs/poor management and different biotic/abiotic stresses (climate, diseases...).
- Farmers need training and/or inputs to fully exploit potential. This can be achieved by setting up demonstration plots and/or providing inputs, such as fertilizers.
- Though it is important that breeders' trials reflect current farmer practices and constraints, it is also important to take future developments into account, i.e. intensive vs. extensive production, fertilizer/agrochemical usage, clonal vs. seedling varieties, adequate planting densities, climate change scenarios, and rootstock and pollination compatibility.
- The surveys indicated variation in availability of improved varieties, partly due to the proximity of seed gardens.
- There is a need to develop technology for clonal gardens, especially in West Africa.
- The potential/economics of new technologies, e.g. tissue culture as a mean to distribute new varieties needs to be established.

- Consideration is to be given to mixtures of clones supplied at farm and region levels.
- Farmers have agreed to make their materials available to other farmers in the current project. However, intellectual property rights (IPR) including farmers' rights with regard to farm accessions have to be given further attention.

Session 1.2. On-farm trials established and evaluated

Topics

- Trial design
- Number of plots established and still active
- Results from older on-farm trials
- Constraints and perspectives.

Main objectives

- To compare the performance of improved hybrids selected on-station with farmers' selections.
- To involve the farmers in the selection process of improved and high-yielding cocoa varieties.
- To facilitate the adoption of improved cocoa varieties by farmers.

Conclusions and recommendations

- Farmers' selections can perform as good as or even better than improved varieties, and thus could be used in breeding activities.
- On-farm trials are positive but not always easily conducted.
- Expectations for farmers and scientists should be made clear from the beginning of the project and scientists should stay in regular contact with farmers.
- The combination of on-farm trials with farmers' capacity building is recommended.
- The involvement of extension officers is recommended in all the steps of the on-farm trials for more efficiency.
- The methodology should be simplified and adapted to the farmers' skill.
- The selection of key farmers is recommended.
- The simplified method based on the counting of pods at the beginning of the main harvest season on the cocoa tree would be a more adapted alternative method for evaluating yields on-farm.
- The use of fertilizer in on-farm trials is recommended, but there is a need to provide funds in the initial budget.
- The distance between the farmers' farms and the research station can be a reel constraint.
- The lack of chupons, and of uniformity of chupons, is a constraint to the basal chupon grafting technique, increasing within-clone variation.
- Collaboration between breeders and the other specialists (agronomist, pathologists, entomologists, etc.) is recommended.

Perspectives

- Continue data collection in the various trials, taking into account resistance to insects and diseases, pod and bean characteristics, as well as fat content.
- Promising cultivars should be established in demonstration plots to validate their performance to farmers.

- Demonstrations plots could be linked to a multiplication effort according to the type of planting material to be distributed.
- Development of extension materials should be tailored to local needs.

Session 2. Molecular marker studies of farm accessions

Topics

- Comparison of genetic diversity in African farmers' fields with breeding collections
- Conclusions of results on breeding
- Quantitative trait loci (QTL) studies carried out by USDA.

Conclusions and recommendations

- High incidence was observed of non-true-to-type improved planting material in farmers' fields (traditional germplasm has been replaced by planting material with improved origins).
- Diversity among farm accessions is largely comprised within the diversity used by breeders and is different among countries.
- Diversity in genebanks is low proportionally to the existing accessible diversity available in the species.
- Since a low number of reference clones were used in these studies, it appears to be a differentiation between Upper and Lower Amazon clones. This is due to the fact that traditional cultivars are much less diverse. It is necessary to be more specific when referring to genetic backgrounds. Use of the new classification will help.
- It was proposed to integrate the farmers' accessions data for a study combining all the four data sets presented.
- Efforts are being made using molecular markers to identify cultivars that could be marketed according to their genetic and geographic origin (Ecuador). This could create added value. The new classification could help in this direction.
- Preventive breeding for moniliasis and witches' broom resistance seems to be an important concern. Two approaches were discussed: marker-assisted selection (MAS) and the introduction of resistant parents; MAS was preferred by some participants as the safest approach.
- Concerns were expressed about funding to characterize the four mapping populations established in the project (two in Costa Rica, one in Côte d'Ivoire and one in Ghana). It was clarified that the current agreements with the institutions concerned should cover the costs of the evaluations.
- The new single nucleotide polymorphism (SNP) technology should allow to shortly map the remaining mapping populations.
- This technology should also improve the rate of application of the molecular biology research.
- It was proposed to have a specific meeting on preventive breeding.
- There was discussion about how to explore the value of the germplasm. Clone evaluations were proposed instead of test crosses, but it was clarified that clones such as GU do not show much value as clones but have been demonstrated to be good parents for yield.

Session 3. Comparative analyses of the International Clone Trial (ICT) results

Topics

- Assessment of stability of agronomic traits and disease resistance
- Assessment of stability of physiological traits
- Evaluation of sensory quality of ICT clones over eight different sites.

Conclusions and recommendations

- While genetic diversity representation and black pod resistance were the main selection criteria at the time of composing the clones for the ICT, it was agreed that yield aspects as well as resistance to other diseases should be considered for future trials.
- The selection of clones for future trials should include taking into consideration under which environmental conditions the performances of clones are to be evaluated.
- The need to include agroclimatic, management practices and soil data along with the evaluation data has been stressed in order to allow a better interpretation of the results.
- Bigger elementary plots per clone could be an improvement.
- The indications that yield plasticity has a significant genetic basis should be confirmed.
- Plant density has been mentioned as a critical aspect that should be considered when assessing the performance of clones over time.
- The opportunity to measure genotype x environment (G x E) interactions for important traits in the ICT could help us to understand the stability of traits better and thus might provide us with research direction with respect to climate change.
- It is suggested to map out how project clones and varieties that derived from them have been used in breeding programmes.

Session 4. Analyses of on-station trials planted in the Germplasm and in the Productivity Projects

Topics

- Analyses of Hybrid Trials, Local Clone Trials, Population Breeding Trials and Regional Variety Trials
- Use of selections for further breeding and as candidate varieties for distribution to farmers
- Constraints and perspectives.

Conclusions and recommendations

- There is a need to continue data collection in the younger trials.
- There is a need for further and more in-depth data analyses.
- It was suggested to identify the pedigrees of all selected varieties.
- Clone names should be standardized in the Final Project Publication (as far as possible in reference to the International Cocoa Germplasm Database (ICGD).
- It was suggested to create a database with raw data to be analysed and USDA-Miami offered to host such a database.

- Fingerprinting data already available as well as further fingerprinting are required to be able to compare data between sites.
- It was suggested to create guidelines for standardized data gathering in cocoa breeding and on how to carry out base calculations.
- Although the first CFC/ICCO/IPGRI project has published Working Procedures, it was noted that the way of calculation of cocoa yield varied between countries. Cumulated, yearly, potential and real yield should be identified correctly to be able to compare data between sites.
- It was suggested to study the variation in yield over years within sites, and also between sites, with local weather data and soil types.
- More information should be available on how to design variety trials comparing clones with seedlings, aiming at avoiding inter-plot interactions.
- There is a need for further funding of the younger trials established with support of the project. A focussed approach is required (prioritizing trials). Governmental funding is to be pursued as well as associations with ongoing projects, aiming at establishing sustainable funding. Funding may be easier for activities near to the farmers and with farmers' involvement.

Session 5. Germplasm enhancement and distribution

Topics

- Progress obtained in the (pre)-breeding programmes in Trinidad and Costa Rica
- Quarantine and distribution of selected accessions
- Perspectives.

Conclusions and recommendations

- Sources of resistance have been identified for black pod and witches' broom using early screening tests and these are being used in pre-breeding and breeding programmes.
- Most of the genes/parents are at international genebanks, available for countries that are interested.
- Several new sources of resistance against *Moniliophthora roreri* were recently identified.
- Some of this material is being used and distributed in Central America for field evaluation and farmers' use.
- More work is required to develop early test against frosty pod rot.
- Constrains to conduct research have been identified and should be taken into account for new plans.
- There are aspects of the host-pathogen-environment relationship that seem to be important to understand better, in order to clarify variations observed between field evaluations and artificial inoculation tests and to standardize early screening tests.
- Research questions have been formulated that may lead the way to continue or start new trials.
- The International Quarantine Centre at Reading (IQCR) has a well-established procedure for transfer of material and for virus indexing which should be maintained and used to minimize this threat.
- Polymerase chain reaction (PCR) methods should be used to improve virus detection at IQCR.

- Somatic embryogenesis as a way to transfer germplasm should be evaluated.
- IPR status of germplasm exchanged should be clarified for future projects.

Session 6. Resistance studies

Topics

- Comparison of alternative testing methods tried during the project
- Conclusions on further use of resistance screening methods.

Conclusions and recommendations

- **Methodologies to assess witches' broom (WB) resistance**
  - Comparisons between the two methods used in Brazil to assess resistance to WB (spray method and agar-droplet method) indicated that both methods are efficient but because of many variations a more practical method needs to be developed, although the spray method seems to be easier to use.
  - The diameter of the broom base to assess resistance to WB is used in Trinidad and Venezuela, but not in Ecuador and Brazil because of the variation in symptoms' expression between these countries.
  - Need to standardize screening methods so as to obtain a faster method that can be used with confidence for evaluation of resistance to WB (laboratory test, seedlings test, field observation).

- **Mirid antixenosis, tolerance and resistance**
  - Antixenosis was correlated with cumulated damage caused by mirids in the field
  - Tolerance to mirids might be explained by the tolerance to the *Lasiodiplodia theobromae* fungus.

- **Existence of several types (at least three) of the clone PA 150**
  - There is a need to focus further research on this issue (comparison of all the types of PA 150).

- **Promising clones for resistance**
  - The clone IMC 47 seems to show combined resistance to black pod, witches' broom and *Ceratocystis*. Further resistance studies need to be focused on this clone and on other promising clones (e.g. French Guiana populations).

- **Use of molecular markers for resistance studies**
  - In Brazil, molecular markers are very important for the breeding programme.
  - In Nigeria and Cameroun, molecular markers appear to show associations with resistance to black pod disease.

Session 7. Impact and perspectives of project activities

Topics

- Perceived benefits and impacts of the CFC project activities
- Needs for continuation of ongoing activities
- Proposals for new collaborative activities.

Content of Session 7

Four presentations were given:

1. Views as perceived by the co-financing institutions
2. Compilation of inputs received from project partners on major benefits perceived, needs for continued actions, and identification of new areas of activities
3. Proposal from Brazil for a new international clone trial and pre-breeding for disease resistance
4. Presentation of a format for the final project publication.

Conclusions and recommendations

- **1. Views as perceived by the co-financing institutions**

  - As main issues for sustainable cocoa-growing were identified: pests and diseases, planting materials, and soil fertility.
  - As quality criteria were identified: bean size and uniformity, fat content, flavour and other organoleptic quality traits.
  - The empowerment of farmers is one important issue in addressing the production constraints.
  - Distribution of planting materials is a new initiative with cocoa industry involvement (Nestlé and Mars Inc. in Côte d'Ivoire).
  - In summary: more cocoa is needed, closer links with farmers are required, decrease of cocoa quality is to be avoided through specifications and traceability, building capacity on how to supply cocoa farmers with improved planting materials, and sector capacity building.
  - Industry priority with regard to environmental services of cocoa? The author agreed and identified two areas that are being dealt with: pesticide rationalization and cultural control of diseases and pests.
  - The aim of industry is that cocoa should be a profitable crop, avoiding the move of farmers to other crops.
  - Dark chocolates could be the way forward to increase consumption, but yet with reduced impact (1-2% of world market), as well as chocolates with increased health benefits (with increased anti-oxidants).
  - The Cocoa of Excellence project was mentioned as an initiative promoting superior cocoa origins. Questions to be answered are sustainability of this initiative as well as industry support for it.

- **2. Compilation of inputs received from project partners on major benefits perceived, needs for continued actions, and identification of new areas of activities**

  - Among the benefits, the distribution of selected materials should be given due attention. It was argued however, that distribution of selected materials should not be done by breeders but by specialized institutions.
  - Research agenda setting was identified as an additional benefit of the project, as well as mirid resistance studies in West Africa and the setting up of the Asian Cocoa Breeders Group.
  - Needs for continued activities were discussed briefly. Setting up of a new clone trial would need to consider use of well-selected clones.
  - The rationale for setting up new projects should be identified.

- **3. Proposal from Brazil for a new international clone trial and pre-breeding for disease resistance**

  - The main topic of the Brazilian proposal is to start new regional/international trials and preventive breeding activities aiming at creating varieties with resistance to alien diseases and pests.
  - The rationale for the new project are the high crop losses due to spreading diseases and pests, the quantitative nature of the resistances as well as the availability of resistance to major diseases and pests.
  - Brazil is willing to make available selected varieties with multiple resistances to witches' broom in populations created specifically for this purpose. This is of crucial importance as resistance of the Scavina source has been overcome in Brazil and also elsewhere.
  - The following topics are part of the proposed activities:
    - Exchange of resistant accessions within and between continents,
    - Crosses with local clones, followed by family selection and new clone selection,
    - Molecular marker studies on the diversity of the fungus, and on associations with disease resistance,
    - Selection of superior clones,
    - Training of scientists in new technologies,
    - Establishment of a basis for a global breeding approach.

  - Due attention is to be paid to existing restrictions to move germplasm through quarantine.
  - Amazon materials that could be made available have shown good resistances to WB, black pod and *Ceratocystis*.
  - It is important to get feedback from the countries. Comments from the countries should be taken into account in the proposal.
  - It was suggested to start with setting up a clone trial in the Americas and include materials selected in the Costa Rica and Trinidad pre-breeding programmes.
  - The advantage of the proposal in providing new genes for resistance to alien diseases for Africa was highlighted.
  - The complementarity of the proposal with regard to the CFC/ICCO/Bioversity Projects was underlined.

- **4. Presentation of a format for the final project publication**

  - Points to be included in the final project publication are:
    - What are the direct outputs of the project,
    - When farmers would have access to new varieties, and
    - A table summarizing the main results of the project.

  - The timeframe to publish the final project publication was set to be before the end of 2010, with draft articles to be provided over the next 2 months.

Appendix II. Programme

Collaborative and Participatory Approaches to Cocoa Variety Improvement
Final Workshop of the CFC/ICCO/Bioversity Project on
"Cocoa Productivity and Quality Improvement: a Participatory Approach"
Organized by the Ghana Cocoa Board and Bioversity International
31 May-4 June 2010, Accra, Ghana

Monday, 31 May

08:00 Registration

Opening Session

Convener: Chief Executive of the Ghana Cocoa Board (Mr Anthony Fofie)

08:30 Welcome words (Representatives of CFC, ICCO, COPAL, Bioversity International, Co-financing Institutions and the Ghana Cocoa Board)

09:00 Coffee break

Session 1. Salient features of the farm survey and of the on-farm trials

Session 1.1. Salient features of the farm survey and collecting of interesting trees

Chairman: Jan Engels Secretary: Michelle End

Topics:

- *Knowledge of farmers on their planting materials*
- *No. of interesting trees identified*
- *No. of trees established in Farm Selection Observation Plots (FSOPs)*
- *Evaluation of farm selections for disease resistance*
- *Use of selected trees in on-farm trial plots*

09:30 Introductory notes (Bertus Eskes, Bioversity International)
10:00 Côte d'Ivoire (Mathias Tahi, CNRA)
10:10 Ghana (Stephen Opoku, CRIG)
10:20 Cameroon (Bruno Efombagn, IRAD)
10:30 Nigeria (Peter Aikpokpodion, CRIN)
10:40 *Questions and answers*
10:50 *Discussion and conclusions*
11:10 Trinidad (Kamaldeo Maharaj, MALMR)
11:20 Ecuador (Freddy Amores, INIAP)
11:30 Malaysia (Francis Aloysius, MCB)
11:40 Brazil (Wilson Monteiro, CEPLAC)
11:50 *Questions and answers*
12:00 *Discussion and conclusions*

12:30 Lunch

Session 1.2. On-farm trials established and evaluated

Chairman: Ray Schnell Secretary: François N'Guessan

Topics:

- *Trial design*
- *No. of plots established and still active*
- *Results from older on-farm trials*
- *Constraints and perspectives*

14:00	Côte d'Ivoire (Mathias Tahi, CNRA)
14:15	Cameroon (Bruno Efombagn, IRAD)
14:30	Nigeria (Peter Aikpokpodion, CRIN)
14.45	*Questions and answers*
15:00	*Coffee break*
15:30	Brazil (Wilson Monteiro, CEPLAC)
15:45	Ecuador (Fredy Amores, INIAP)
16:00	Trinidad (Kamaldeo Maharaj, MALMR)
16:15	Venezuela (Olga Movil, INIA)
16:30	*Questions and answers*
16:45	Malaysia (Francis Aloysius)
17:00	Papua New Guinea (Jeffrie Marfu, CCI)
17:15	*Questions and answers*
17:30	*Discussions and conclusions*
19:30	***Welcome cocktail (offered by Mars Inc. and CFC)***

Tuesday, 01 June

Session 2. Molecular marker studies of farm accessions

Chairman: Wilbert Phillips Secretary: Juan Carlos Motamayor

Topics:

- *Comparison of genetic diversity in farmers' fields with breeding collections*
- *Conclusions of results on breeding*
- *QTL studies carried out by USDA*

08:30	Genetic diversity of farm accessions in Cameroon (Bruno Efombagn, IRAD)
08:45	Genetic diversity of farm accessions in Côte d'Ivoire (Désiré Pokou, CNRA)
09:00	Genetic diversity of farm accessions in Nigeria (Peter Aikpokpodion, CRIN)
09:15	Genetic diversity of farm accessions in Ghana (Stephen Opoku, CRIG)
09:30	*Discussion and conclusions*
09:45	QTL studies in Miami (Ray Schnell, USDA)
10:00	*Discussion and conclusions*
10:15	*Coffee break*

Session 3. Comparative analyses of the International Clone Trial (ICT) results

Chairman: Martin Gilmour Secretary: Jan Engels

10:45	Comparative analyses of agronomic traits and disease incidence (Bertus Eskes, Bioversity/CIRAD)
11:00	*Questions and answers*
11:10	Comparative analyses of physiological traits (Andrew Daymond, Reading University)
11:25	*Questions and answers*
11:35	Comparative analyses of sensory traits (Christian Cilas and Fabienne Ribeyre, CIRAD)
11:50	*Questions and answers*
12:00	*Discussion and conclusions*
12:30	*Lunch*

Session 4. Analyses of on-station trials planted in the Germplasm and in the Productivity Projects

Chairman: Dario Ahnert Secretary: Bertus Eskes

Topics:

- *Analyses of Hybrid Trials, Local Clone Trials, Population Breeding Trials and Regional Variety Trials*
- *Use of selections for further breeding and as candidate varieties for distribution to farmers*
- *Constraints and perspectives*

14:00	Trinidad (Patricia Maharaj, MALMR)
14:15	Costa Rica (Wilbert Phillips and Carlos Astorga, CATIE)
14:30	*Questions and answers*
14:45	Malaysia (Kelvin Lamin, MCB)
15:00	Papua New Guinea (Jeffrie Marfu, CCI)
15:15	*Questions and answers*
15:30	*Coffee break*
16:00	Nigeria (Peter Aikpokpodion, CRIN)
16:20	Peru (Luis Garcia Carrion, UNAS)
16:40	*Questions and answers*
17:00	*Discussion and conclusions*

Wednesday, 02 June

Session 4. Continued

08:30	Côte d'Ivoire (Mathias Tahi, CNRA)
09:00	Ghana (Francis Padi, CRIG)
09:30	*Questions and answers*
09:45	*Coffee break*
10:15	Brazil (Wilson Monteiro, CEPLAC)
10:45	Ecuador (Freddy Amores, INIAP)

11:15	Venezuela (Olga Movil, INIA)
11:30	*Questions and answers*
11:45	*Discussion and conclusions*
12:30	*Lunch*

Session 5. Germplasm Enhancement and Distribution

Chairman: Edna Dora Luz Secretary: Carmen Suarez

Topics:
- *Progress obtained in the (pre)-breeding programmes*
- *Quarantine and distribution of selected accessions*
- *Perspectives*

14.00	Black pod and monilia resistance at CATIE (Wilbert Phillips)
14:20	Black pod resistance at CRU (Surendra Maharaj)
14:40	Witches' broom resistance at CRU (Surendra Maharaj)
15:00	*Questions and answers*
15:10	*Discussion and conclusions*
15:30	Quarantine and distribution of selected germplasm at Reading University (Andrew Daymond)
15:45	*Discussion and conclusions*
16:00	*Coffee break*

Session 6. Resistance studies

Chairman: Bertus Eskes Secretary: Salomon Nyassé

Topics:
- *Comparison of alternative testing methods tried during the project*
- *Conclusions on further use of resistance screening methods*

16:30	Methodologies to assess witches' broom resistance at CRU (Surendra Maharaj, CRU)
16:50	Methodology to assess witches' broom resistance at INIAP (Carmen Suarez, INIAP)
17:10	*Discussion and conclusions*

Thursday, 03 June

Session 6. Continued

08:30	Methodology to assess witches' broom resistance at CEPLAC (Edna Dora Luz, CEPLAC)
08:50	*Discussion and conclusions*
09:00	Results obtained on mirid antixenosis, tolerance and resistance at CNRA (François N'Guessan)
09:20	*Discussion and conclusions*
09:30	*Coffee break*

Session 7. Impact and perspectives of project activities

Chairman: Stephan Weise Secretary: Bertus Eskes

Topics:

- *Perceived benefits and impacts of the CFC project activities*
- *Needs for continuation of ongoing activities*
- *Proposals for new collaborative activities*

10:00	Views as perceived by the Co-financing institutions (Martin Gilmour, Mars Inc.)
10:15	*Discussion and conclusions*
10:45	Compilation of inputs received from project partners (Stephan Weise, Bioversity International)
11:00	*Discussion and conclusions*
11:30	Proposal from Brazil for a new International Clone Trial (Dario Ahnert, UESC, Brazil)
12:00	*Discussion and conclusions*
12:15	Outline for the Final Project Publication (Bertus Eskes, Bioversity International)
12:30	*Lunch*

Session 8. Presentations of conclusions

Chairman: Yunusa Abubakar Secretary: Stephan Weise

14:00	Presentations of 5 minutes by the secretaries of each of the eight sessions followed after each presentation by 5 minutes of questions and answers.
15:20	*General discussion and conclusions*
16:30	*Coffee break*

Closing Session

Convener: Director of Economics and Statistics Division of ICCO (Dr Jean-Marc Anga)

17:00	Closing remarks (Representatives of Bioversity International, Co-financing Institutions, ICCO and Ghana Cocoa Board)
19:30	*Workshop Dinner (offered by the Cocoa Board of Ghana)*

Friday, 4 June

07:30-17:00 Field visit to the Cocoa Research Institute of Ghana (Tafo)

Appendix III. List of participants

Collaborative and Participatory Approaches to Cocoa Variety Improvement
Final Workshop of the CFC/ICCO/Bioversity Project on
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31 May-4 June 2010, Accra, Ghana

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Appendix IV. Full names and acronyms of project partners

National agricultural research institutions (NARS)

- Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica
- Cocoa Research Institute of Nigeria (CRIN), Nigeria
- Cocoa Research Institute of Ghana (CRIG), Ghana
- Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), Brazil
- Instituto Nacional de Investigaciones Agrícolas (INIA), Venezuela
- Centre National de Recherche Agronomique (CNRA), Côte d'Ivoire
- Instituto Nacional de Investigaciones Agropecuarias (INIAP), Ecuador
- Institut de Recherches Agronomiques pour le Développement (IRAD), Cameroon
- Malaysian Cocoa Board (MCB), Malaysia
- Ministry of Agriculture, Land and Marine Resources (MALMR), Trinidad and Tobago
- PNG Cocoa & Coconut Institute (CCI), Papua New Guinea
- Universidad Nacional de la Selva (UNAS), Peru

International agriculture research institutes (IARI)

- Centre de Coopération Internationale en Recherches Agronomiques pour le Développement – Département des Systèmes Biologiques (Cirad-Bios), France
- Cocoa Research Unit (CRU), Trinidad and Tobago
- United States Department of Agriculture (USDA), USA
- University of Reading, Reading, UK

Co-financing organizations

- Cocoa Research Association (CRA), UK (formerly Biscuit, Cake, Chocolate and Confectionery Association or BCCCA)
- CIRAD, France
- Guittard Chocolate Company, USA
- Mars Inc. Company, UK and USA
- United States Department of Agriculture (USDA), USA
- World Cocoa Foundation (WCF), USA

Supervisory Body

- International Cocoa Organization (ICCO), London, UK

Project Executing Agency

- Bioversity International (or Bioversity), Italy, through the Project Coordination Unit which is part of the Commodities for Livelihoods Programme (CfL), Montpellier, France

Main Financing Institution

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